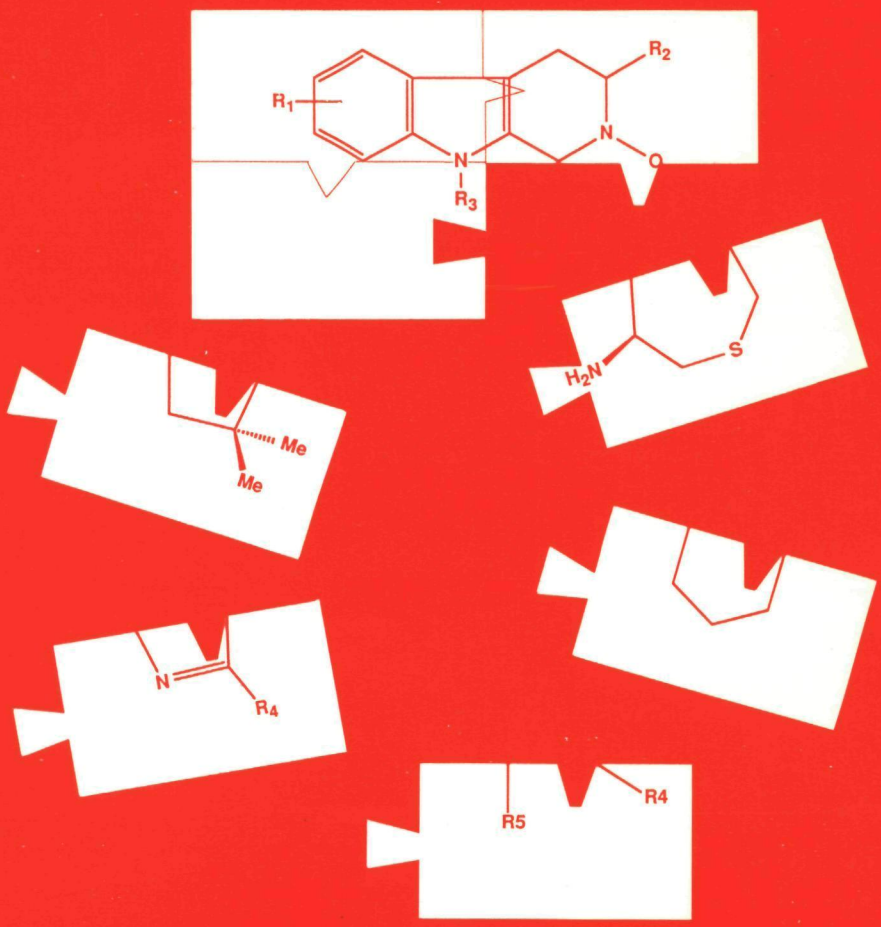
N-HYDROXY-β-CARBOLINES

Syntheses, Applications, and Biological Activities



Pedro H.H. Hermkens

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Syntheses, Applications, and Biological Activities

EEN WETENSCHAPPELIJKE PROEVE OP HET GEBIED VAN

DE NATUURWETENSCHAPPEN

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR AAN DE KATHOLIEKE UNIVERSITEIT TE NIJMEGEN VOLGENS BESLUIT VAN HET COLLEGE VAN DECANEN IN HET OPENBAAR TE VERDEDIGEN OP DONDERDAG 21 JUNI 1990 DES NAMIDDAGS TE 3.30 UUR

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Co-promotores: Dr. J.W. Scheeren

Dr. C.G. Kruse

The investigations the results of which are presented in this thesis were carried out in the Department of Organic Chemistry, University of Nijmegen, Nijmegen, The Netherlands.

These studies were carried out under the auspices of the Technology Foundation of the Netherlands (STW, project no. NCH57.0825) with financial aid from the Netherlands Organization for Scientific Research (NWO). The receptor studies reported in the last chapter of this thesis were carried out at Duphar Research Laboratories, Weesp.

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Aan mijn Ouders

Voor Miranda, Niels en ?

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Pedro

CONTENTS

CHAPTER

CONTENTS

CHAPTER

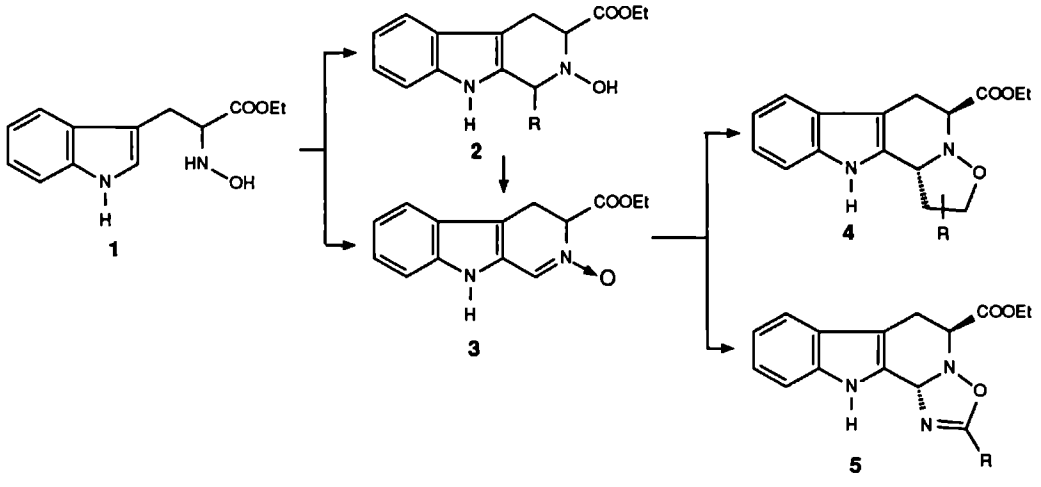
Chapter 1

GENERAL INTRODUCTION

In the past decade pioneering research in the field of N-hydroxy amino acids -by amongst others the Nijmegen group[1,2]- showed the biogenetic as well as the synthetic relevance of this class of compounds. In particular N-hydroxytryptophan (**1**) was studied in some detail.[2] In approaches to several natural products, *e.g.* sporisdesmins, neoechinulins and fumitremorgins it appeared that **1** is an excellent precursor for α-functionalized-, α,β-functionalized- as well as α,β-dehydro-tryptophan derived compounds.

It was also demonstrated that N-hydroxytryptophan can be converted into β-carbolines in two ways (Scheme I). A Pictet-Spengler reaction of **1** with acetals provides the 1,3-disubstituted N(2)-hydroxy-1,2,3,4-tetrahydro-β-carbolines (**2**).[2d] A modified Bischler-Napieralski reaction of **1**

Scheme I

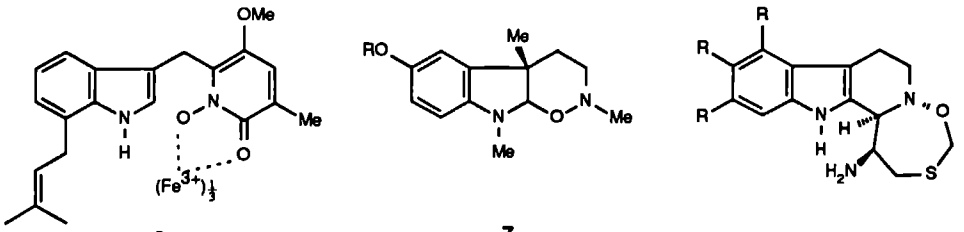


with trimethylorthoformate gives N(2)-oxo-3,4-dihydro-β-carboline (**3**) of which the nitrone function can undergo 1,3-dipolar cycloaddition reactions with alkenes[2e] and nitriles[2f], providing the isoxazolidine (**4**) and the Δ^4-1,2,4-oxadiazoline (**5**) annulated tetrahydro-β-carbolines, respectively. Both approaches gave an entrance to a new class of compounds, the N-oxy-β-carbolines.

This thesis describes the in depth continuation of this research, with emphasis on substituted tryptamines. Further research on this topic was considered to be of interest because of the following reasons:

i) Secondary metabolites occur featuring a N-hydroxylated tryptophan moiety *e.g.* Astechrome[3] (**6**)

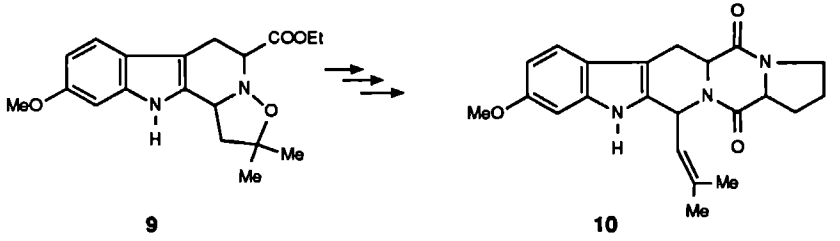
Scheme II



or containing the skeleton of N-hydroxytryptamine *e.g.* Geneserine[4] (**7**). Moreover, the secondary metabolites belonging to the class of the Eudistomins (**8**) (Scheme II) are a synthetic challenge.

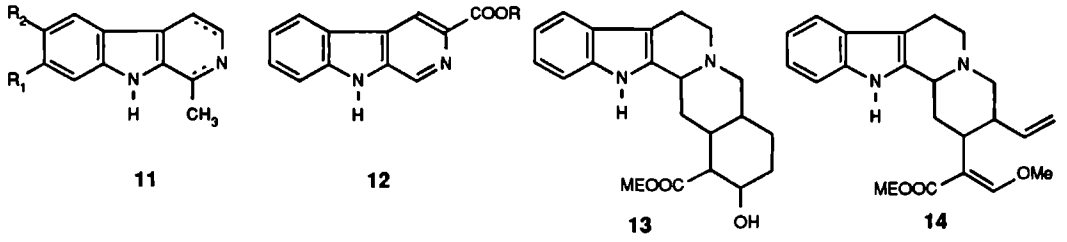
ii) Previously, the N-O functionality of N-hydroxytryptophan has been used for the synthesis of α-functionalized-, α,β-functionalized- and α,β-dehydro-tryptophan derivatives.[1,2] We now wondered whether transposition of the N-O-functionality towards other positions of a molecule would be feasible. For instance, we reasoned that the isoxazolidine **9** might be a suitable precursor for the fumitremorgin series (*i.e.* **10**, Scheme III)

Scheme III



iii) β-Carbolines and tetrahydro-β-carbolines[10] have a broad and interesting pharmalogical profile. Harman-like β-carbolines, *e.g.* **11** (Scheme IV) have shown affinities for the tryptamine[11-15] and

Scheme IV

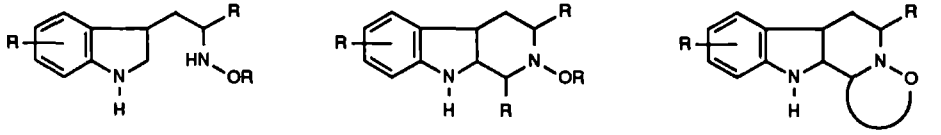


serotonin[16-18] receptors and the 3-carboxylated β-carbolines (*e.g.* **12**) bind with high affinities to the benzodiazepine receptor.[19-37] Moreover, the therapeutic activity of several indole alkaloids which

contain the tetrahydro-β-carboline fragment such as yohimbine- (*e.g.* **13**), and corynatheine-like (*e.g.* **14**) derivatives has been demonstrated.[38]

Chemical modification of β-carbolines and tryptamines -which form the backbone of the β-carbolines- continues to be the object of intensive study in order to find new drugs. The relatively

Scheme V



15 **16** **17**

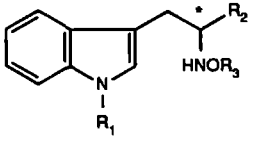
unknown N-oxy-tryptamines and N-oxy-β-carbolines (*e.g.* **15-17**, Scheme V) constitute an interesting new line in this pharmacological field. As the pharmacodynamic interaction of the basic nitrogen plays a key-role at the receptor level, it might be valuable to study the influence of an electronegative oxygen adjacent to this nitrogen in these structures. Alterations in basicity, nucleofilicity, polarization, steric hindrance and additional H-bridge formation might be considered in this context. Obviously, the information obtained will lead to more insight on the molecular level concerning the critical parameters involved in receptor affinity and consequently to the development of new pharmacotherapeutics.

Finally, of special interest are also the above-mentioned eudistomins (**8**). They display potent antiviral activity[5-9] against *Herpes simplex* Type 1 (HSV-1) and *Polio* vaccine Type 1 viruses and antitumour activity.[8,9]

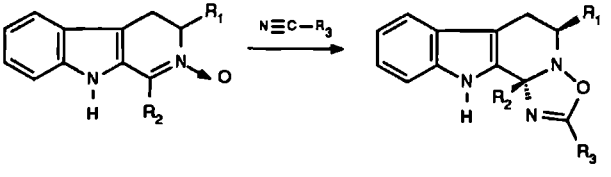
Outline of this Thesis

In this thesis the following topics are discussed:

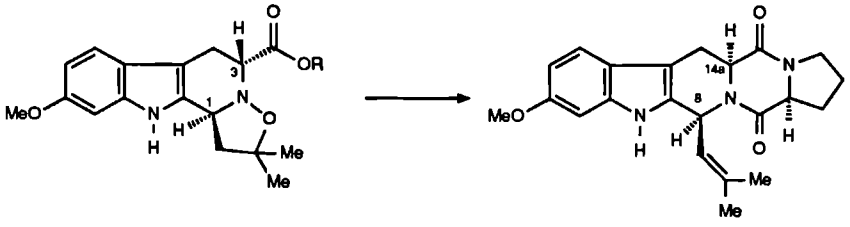
- The synthesis of new N-oxy-tryptamines and an approach to optically pure N-hydroxy-tryptophan derivatives.



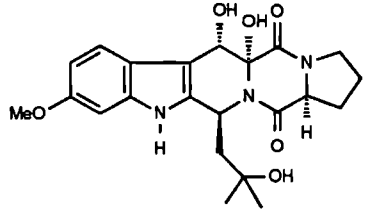
- The synthesis of β-carboline nitrones and the study of the mechanism and scope of the nitrone-nitrile 1,3-dipolar cycloaddition.



- The first total syntheses of fumitremorgin C and verruculogen TR-2 starting from N-oxo-β-carbolines.

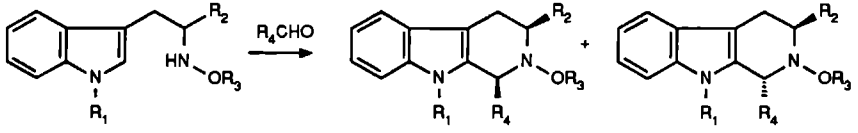


Fumitremorgin C

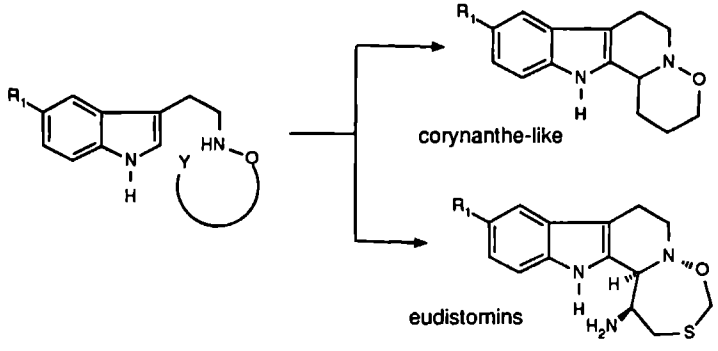


Verruculogen TR-2

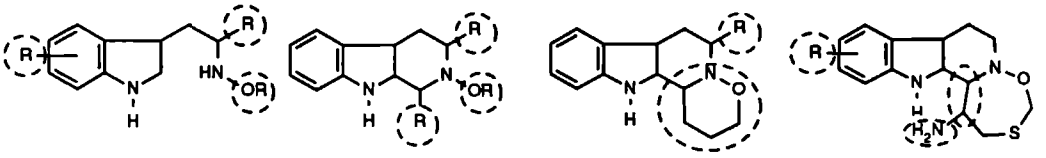
- The synthesis of several substituted N-hydroxy(alkoxy)-tetrahydro-β-carbolines using the intermolecular Pictet-Spengler reaction of N-hydroxy(alkoxy)-tryptamines and -tryptophans with aldehydes and the study of the influence of the N-O substituent on the stereoselectivity of this reaction.



- The synthesis of tetracyclic N-oxo-β-carbolines having structural resemblances to eudistomins or corynanthe-like alkaloids by a novel, intramolecular Pictet-Spengler reaction of N-alkoxy-tryptamine or -tryptophan derivatives.



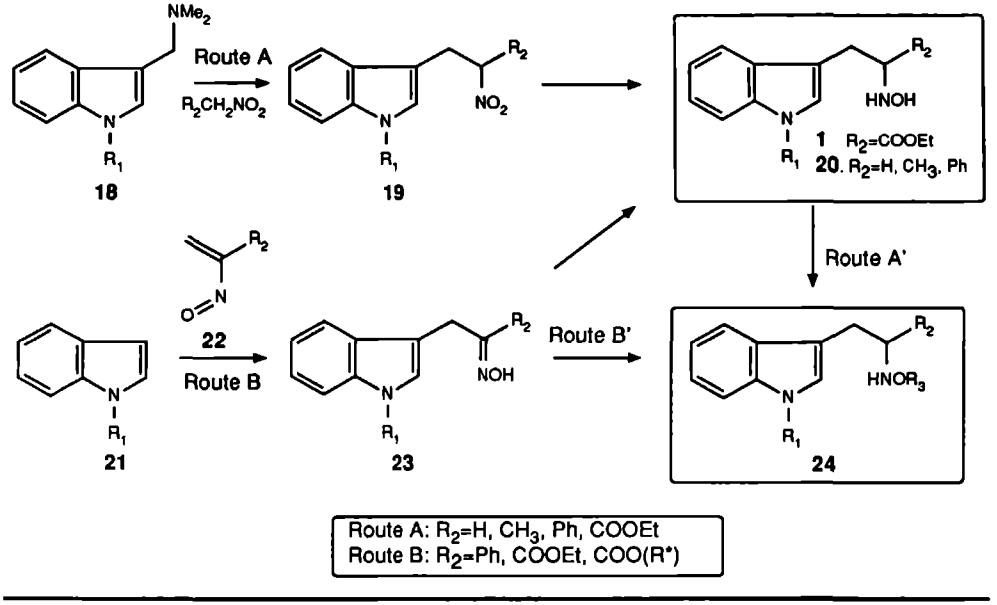
- The structure-activity relationship (SAR) study of N-oxy-tryptamines and -β-carbolines with respect to the benzodiazepine, serotonin and tryptamine receptors and the SAR study of eudistomins and analogues with respect to their antitumour and antiviral activity.



Introduction to the chapters

In chapter 2 an efficient route to N-hydroxytryptophan (**1**) and -tryptamine (**20**: R_2=H, Me, Ph) derivatives is described proceeding *via* the corresponding nitro compounds **19** prepared from the easily accessible gramine **18** and nitromethane derivatives[39] (Scheme VI, Route A). Reduction of the nitro group with Al-amalgam gives the N-hydroxy compounds in high yields.

Scheme VI



A second route features the well-known approach *via* the oxime compounds **23**, which are prepared by a cycloaddition reaction of indole derivatives (**21**) and nitroso olefins (**22**) (Scheme VI, Route B).[2] Reduction of the oxime double bond is accomplished with borane-trimethylamine complex ($Me_3N.BH_3$) under acidic conditions. This route seems to be only efficiently applicable if strongly electron-withdrawing groups R_2 are present in the nitroso-olefin **22** (*e.g.* R_2=COOEt).

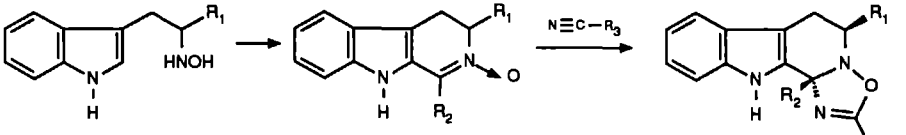
N-Alkoxy-tryptamines (**24**; R_2=H, Me) and -tryptophans (**24**; R_2=COOEt) can be conveniently prepared by the selective N-protection of **1** or **20** with 2-(trimethylsilyl)ethyl chloroformate, followed by O-alkylation and removal of the TEOC group (Scheme VI, Route A'). N-Alkoxytryptophans (**24**;

R_2=COOEt) can also be obtained by a regioselective O-alkylation of oxime **23** followed by reduction with $Me_3N.BH_3$ (Scheme VI, Route B').

Subsequently, an approach to optically active N-hydroxytryptophan derivatives is described. Diastereoselective reduction of the oxime functionality in the chiral substituted menthol ester derivatives (**23**; R_2=COO(-)-8-phenylmenthyl and COO-(-)-8-naphthylmenthyl) is achieved leading to a derivative of **1** in which the ethyl group is replaced by the corresponding chiral auxiliary (Scheme VI, Route B). *Unfortunately, subsequent removal of the chiral auxiliary failed so far.*

Chapter 3 deals with the synthesis of nitrones (**25**: R_1=COOEt, CH_3, H and R_2=H or R_1=CH_3, R_2=Ph) from the corresponding N-hydroxy compounds (see Scheme VII). Subsequently, the

Scheme VII

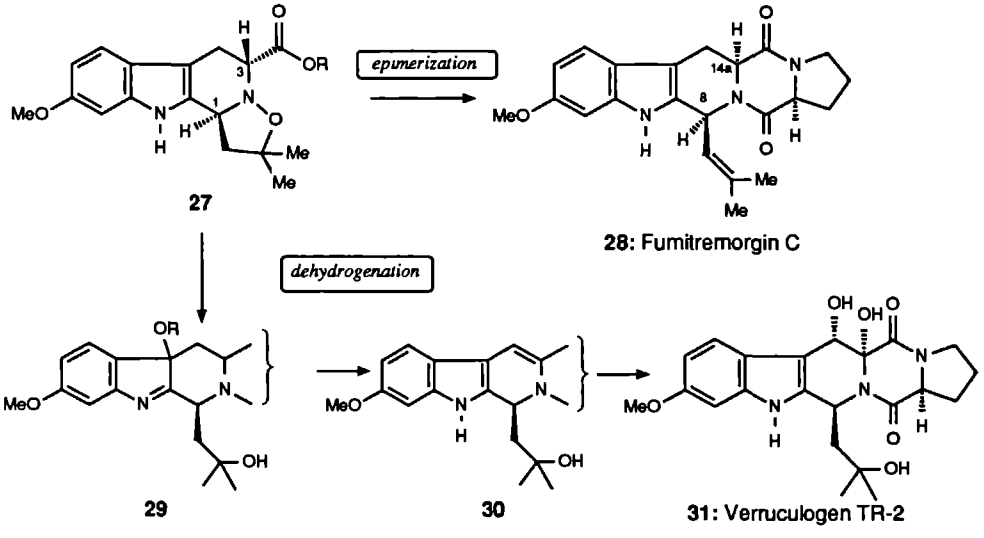


1,3-dipolar cycloaddition of these nitrones **25** with nitriles is discussed. In general, the cycloaddition seemes to be controlled by a HOMO(nitrone)-LUMO(nitrile) interaction, to give Δ^4-1,2,4-oxadiazolines (**26**) with complete regioselectivity. However, a cross-over in the orbital control is observed with strongly electron donating groups R_3. Kinetic studies taking into account solvent polarity and substituent effects (R_3=aryl, Hammett equation) demonstrate that mechanistically this new nitrone-nitrile cycloaddition is consistent with the well-known nitrone-alkene cycloaddition.

In chapter 4 the employment of the isoxazolidine **27** -obtained by cycloaddition of the corresponding nitrone and isobutene in a complete regio- and stereoselective fashion- in the total synthesis of fumitremorgin C (**28**) is highlighted (Scheme VIII). Key-step in this approach is the epimerization of the C(3) carbon to give compounds with a *cis*-relationship (*e.g.* **28**) between the C(1) and C(3) substituents. By completing the synthesis of all four possible stereoisomers -by varying the chirality at C(8) and C(14a) in **28**- we unambiguously determined the configuration at C(14a) of fumitremorgin C as depictet in Scheme VIII (14a(S)). At the onset of our studies the relative stereochemistry at carbon atom 14a of the natural product was unknown.

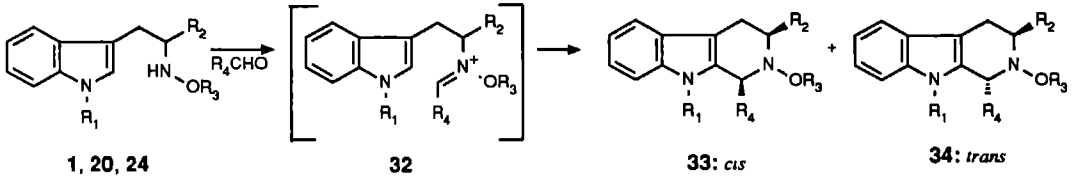
The second half of this chapter deals with the total synthesis of verruculogen TR-2 (**31**). This synthesis features the formal dehydrogenation of tetrahydro-β-carbolines to 3,4-dehydro-β-carbolines (*e.g.* **30**). *This conversion was achieved via the intermediacy of the 3-alkoxyindolenines* **29**, *which* under acidic conditions, rearranged to **30**. The alkene moiety was then converted into a *cis*-diol functionality employing osmium tetroxide.

Scheme VIII



In chapter 5 the synthesis of a wide range of new N-oxo-β-carbolines is reported. The influence of the substituents R_1-R_3 in the N-oxy-tryptophan and -tryptamine derivatives **1**, **20** and **24** on their reactivity in the intermolecular Pictet-Spengler reaction with aldehydes (R_4CHO) to give *cis*- (**33**) and *trans*-β-carbolines (**34**) is studied (Scheme IX). Attention was also paid to the influence of these

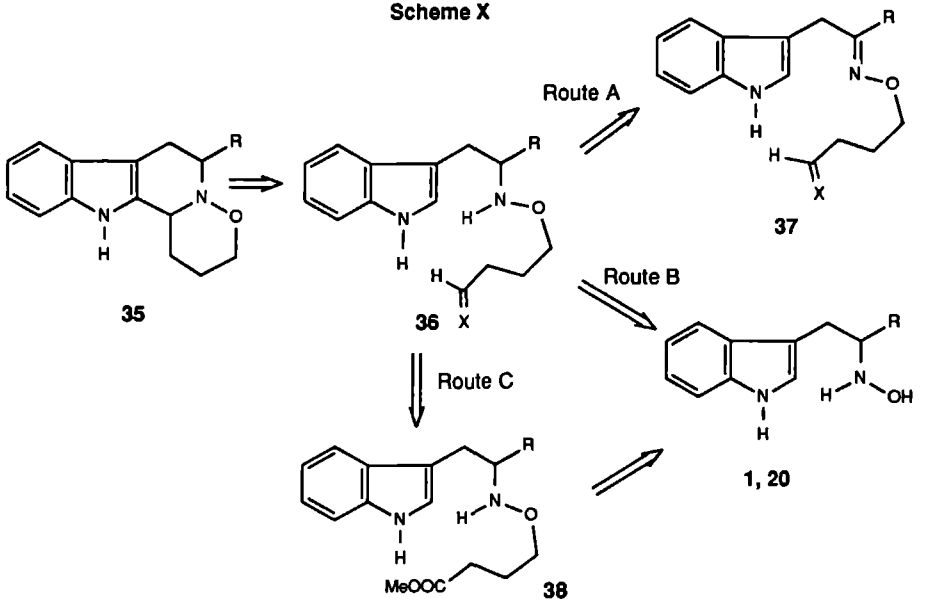
Scheme IX



substituents on the stereochemical outcome of this reaction. The increased electrophilic character of the C=N double bond in the intermediate **32** -due to the oxygen substituent on the nitrogen- increases its reactivity and alters the stereoselectivity, with respect to the deoxy analogues.

Chapter 6 deals with the syntheses of tetracyclic N-oxo-β-carbolines **35** (R=H, CH_3, COOEt), analogues of the corynantheine indole alkaloids.These target molecules could be obtained by a novel intramolecular Pictet-Spengler reaction employing **36** (Scheme X). This crucial intermediate **36** represents a N-alkoxytryptamine derivative having an aldehyde function or a masked derivative thereoff in δ-position of the alkoxy chain. Three approaches were studied towards **36**. Route A

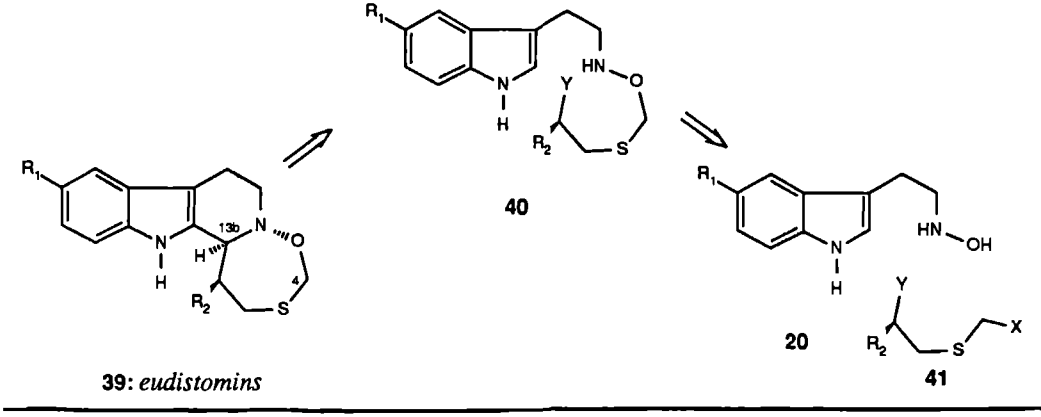
Scheme X



features a selective reduction of the O-alkylated oxime function present in **37**. In route B a selective O-alkylation of the N-hydroxy compounds **1** and **20** with a properly functionalized four carbon substrate is carried out. Route C consists of a selective reduction of the ester function in **38**, generated from **20**. The most effective approaches are routes B and C, whereas route A gave poor results.

In chapter 7 the application of the intramolecular Pictet-Spengler reaction in the total synthesis of eudistomin analogues **39** is described. First, model-studies resulted in the the synthesis of the

Scheme XI



eudistomin skeleton **39** (R_1=R_2=H). Intramolecular Pictet-Spengler reaction of N-alkoxytryptamines

40 (R_1=R_2=H, Y=CH(OMe)$_2$ or Y=COOMe) -derived from **20** and the chloromethylsulfide derivative **41**- gives **39**.

In the second half of this chapter the syntheses of all four stereoisomers of the eudistomins **39** (R_2=NH_2 and R_1=H or OMe) are described. In this case the intramolecular Pictet-Spengler reaction is only feasible when the ester **40** (Y=COOMe) is used as *in-situ* precursor for the aldehyde (route C, Scheme X).

Chapter 8 deals with structure-activity relationship studies. In the first part the affinities of the previously described N-hydroxy(alkoxy)-tryptophans and -tryptamines (chapter 2), the 3,4-dihydro-N-oxo-β-carbolines (chapter 3), the N-oxo-tetrahydro-β-carbolines (chapter 5) and the tetracyclic N-oxo-tetrahydro-β-carbolines (chapter 6) for the tryptamine-, serotonin- and benzodiazepine-receptors are given. In general the N-oxo substituent abolishes the affinity, though within some N-oxo derivatives a rather selective profile is observed. These receptor studies were performed in Duphar Research Laboratories, Weesp.

The second half of this chapter deals with the structure-activity relationships of several eudistomin analogues with regard to their antitumour and antiviral activity. These antitumour assays were performed by Peter Lelieveld (TNO-CIVO institutes, Zeist) and the antiviral assays by Prof. E. DeClercq (Rega institute, Leuven).

Antiviral activities

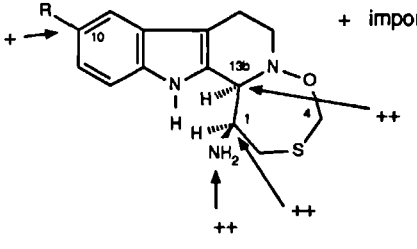
This preliminary SAR study focusses on the influence of the stereochemistry at C(1) and C(13b) and of small alterations of essential structural moieties on the activity.

Seven eudistomin analogues **39** were evaluated on their inhibitory effects on the replication of a variety of viruses including the human immunodefiency virus HIV-1 and HIV-2.

In general we observed with respect to the antiviral activities other than the anti-HIV-1 and -2 activity that *i)* only those stereoisomers are active that have the same configuration at C(1) and C(13b) as the natural products *ii)* the presence of the amino function at C(1) is of importance *iii)* substitution in the indole nucleus alters the potency of the compound.

The results of this preliminary SAR study are summarized in Scheme XII.

Scheme XII. Antiviral and antitumour activity of eudistomin-analogues.



Antitumour activity

Four of the prepared eudistomin analogues are assayed against tumour cells (P388 leukemie) in an *in vitro* clonogenic assay. The preliminary results indicate that the antitumour activity of the compounds tested parallels their antiviral activity as far as the influence of structural parameters is concerned (Scheme XII).

The most potent compound is **39** in which R_1=OMe and R_2=NH_2. This analogue is five times more active in the assays studied than the well-known antitumour compound adriamycine.

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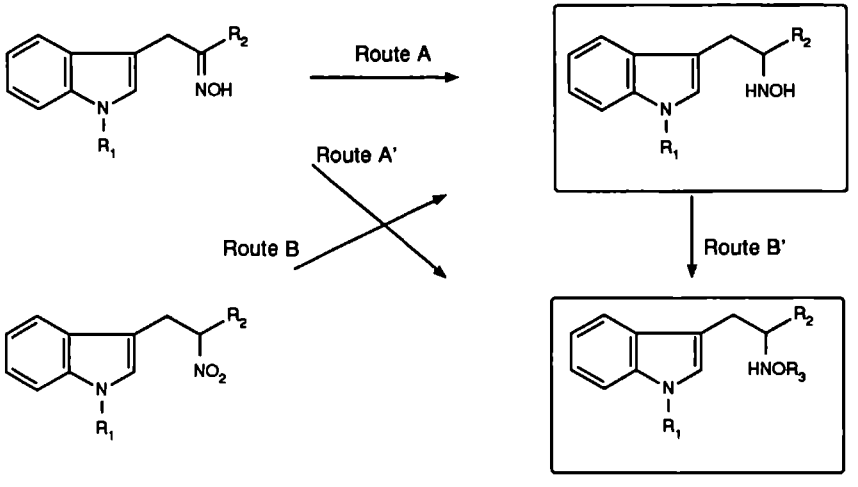
CHAPTER 2

Syntheses of N-hydroxy(alkoxy)-tryptophan and -tryptamine derivatives.



R_2=H, CH_3, Ph, COOEt, COO(R*)

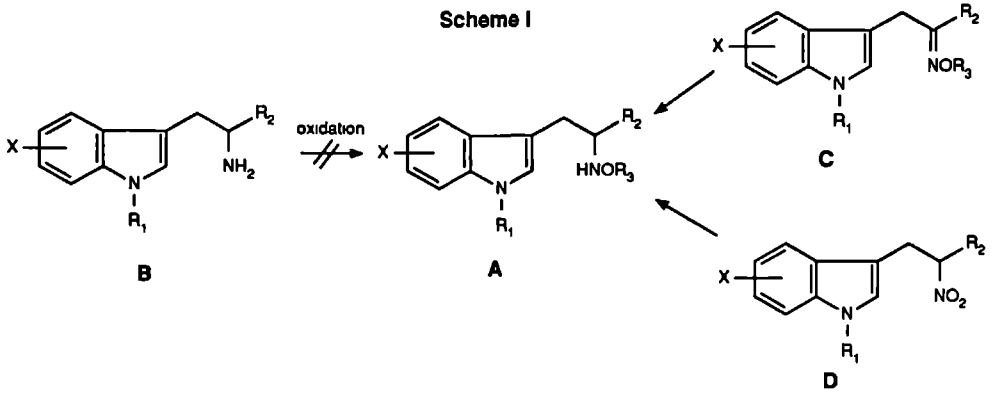
Chapter 2.1

SYNTHESES OF N-HYDROXY(ALKOXY)-TRYPTOPHAN AND -TRYPTAMINE DERIVATIVES.

This chapter intends to provide a survey of the syntheses of N-hydroxy(alkoxy)-tryptophan (Scheme I, **A**, R_2=COOR) and -tryptamine (R_2=H) derivatives. For two reasons we are interested in these compounds. The first has a pharmacological background. The target compounds are derivatives of the neurotransmitters tryptamine and serotonin (5-HT), and it is interesting to study the influence of the N-oxy substitution upon the interaction with the biological receptors. Furthermore, β-carbolines -which can be prepared from tryptophan and tryptamine derivatives[1]- show a broad pharmacological profile. For instance they act as monoamine oxidase inhibitors, 5-HT reuptake inhibitors or they possess strong affinities for the benzodiazepine receptor.[2] Second, the synthetic utility of the title compounds is of importance. N-Hydroxy amino acids in general[3a], and N-hydroxytryptophan[3b,c] in particular not only deserve attention as possible biosynthetic intermediates but also as synthons in the preparation of natural products.[3,4] Natural products containing the N-oxytryptamine moiety such as Geneserine[5] or Eudistomins[6,7] are of special interest in this respect.

The most obvious synthetic method for the N-oxo derivatives (**A**) would be direct oxidation of the amine function in (**B**). However the vulnerability of the indole nucleus towards (electrophilic) oxidating agents precludes this possibility. Since the indole nucleus is relatively stable towards

Scheme I



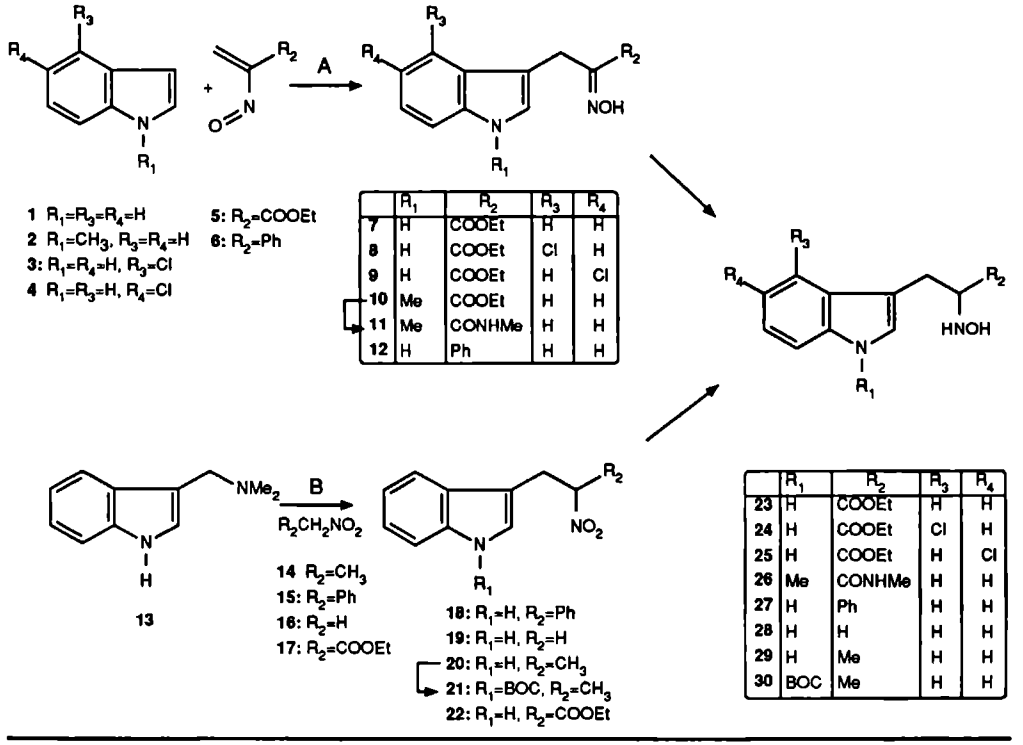
reducing agents, we considered the reduction of oxim derivatives (**C**) and nitro derivatives (**D**).

N-Hydroxy derivatives (Scheme II). The syntheses of N-hydroxytryptophan derivatives like **23** via oxim derivatives (Route A) are well documented[3,4]. Cycloaddition[8] of the nitroso-olefin **5** -prepared

in situ from ethyl α-(hydroxyimino)-β-bromopropanoate- with an excess of **1** gives an adduct which after ring opening and rearomatization affords **7**. Subsequent reduction of the oxime double bond of **7** with borane-trimethylamine complex under acidic conditions gives **23**. In an analogous fashion conjugate addition of 4-chloroindole (**3**) or 5-chloroindole (**4**) to **5** gave the oximes **8** and **9** in 67% and 70% yield, respectively. Reduction with borane-trimethylamine complex afforded **24** (73%) and **25**(94%).

By the same procedure oxime **12** is obtained, from **1** and **6**[9] in only 20% yield. The poor yield is a

Scheme II



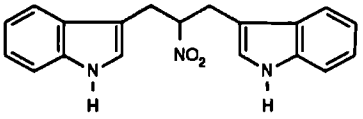
	R_1	R_2	R_3	R_4
7	H	COOEt	H	H
8	H	COOEt	Cl	H
9	H	COOEt	H	Cl
10	Me	COOEt	H	H
11	Me	CONHMe	H	H
12	H	Ph	H	H

	R_1	R_2	R_3	R_4
23	H	COOEt	H	H
24	H	COOEt	Cl	H
25	H	COOEt	H	Cl
26	Me	CONHMe	H	H
27	H	Ph	H	H
28	H	H	H	H
29	H	Me	H	H
30	BOC	Me	H	H

result of the lower reactivity of the nitroso-olefin **6** compared with **5** due to the less electron-withdrawing ability of the phenyl group.[9,10] Reduction of the oxime double bond of **12** failed with borane-trimethylamine complex or Al-amalgam. However, reduction with sodium cyano borohydride in acidic solvent gave the N-hydroxy tryptamine derivative **27** in 95% yield.[11]

Since Route A seems to be only efficiently applicable if strongly electron-withdrawing R_2 groups are present in the nitroso-olefin we studied an alternative route. Heath-Brown *et.al.*[12] prepared the nitro compound **20** (R_2=CH_3) from gramine (**13**) and nitroethane (**14**) by treatment with dimethylsulfate and base. Following the same procedure with α-nitrotoluene (**15**)[13] and nitromethane (**16**) we isolated the nitro compounds **18** (R_2=Ph) and **19** (R_2=H) in 63% and 86% yield, respectively. In the latter case a large excess of nitromethane was necessary in order to suppress the formation of the bisindole

compound **31**, which has not been reported before. Reduction of the nitro functionality of **18-20** into



31

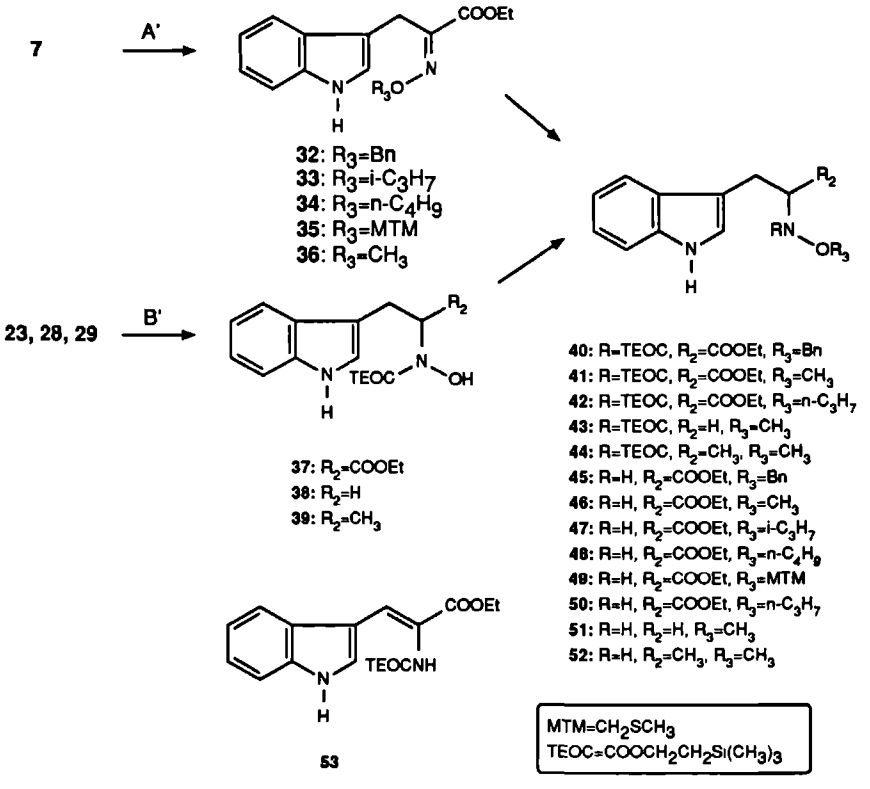
the hydroxylamine group proceeded smoothly with Al-amalgam in ethyl acetate saturated with water to give **27-29** in high to excellent yields.[14] This method appeared to be also suited for the preparation of **23**. By simple heating **13** and ethyl nitroacetate (**17**) in xylene (100°C) the nitro compound **22** was obtained in 90% yield,[15] which by reduction with Al-amalgam afforded **23** in 92% yield.[16] Thus, Route B is a general and a simple approach to both N-hydroxytryptophan and tryptamine derivatives.

We also investigated the preparation of the two indole nitrogen substituted compounds **26** and **30**. The synthesis of **26** has been described (Route A, **2**→**10**→**11**→**26**).[3b] In Route B it was possible to protect the nitrogen of indole with *tert*-butyloxycarbonyl (BOC) in the nitro-stage, following the procedure of Grehn.[17] Reaction of **20** with di-*tert*-butyl dicarbonate in the presence of triethylamine and 4-dimethylaminopyridine in acetonitrile gave **21** in 83% yield and subsequent reduction of the nitro group with Al-amalgam gave **30** in 54% yield.

N-Alkoxy derivatives (Scheme III). One of the approaches we explored for the synthesis of N-alkoxytryptophan derivatives starts with the oxime **7**. (Route A') Alkylation of oximes is known to occur both on oxygen and on nitrogen to give mixtures of oxime ethers and nitrons, respectively.[18] Selective O-alkylation of oximes has been reported using phase transfer conditions[18a], but these conditions failed with **7**.[19] In our hands the method of choice for selective O-alkylation encompasses DMSO as solvent, potassium *tert*-butoxide as base and an alkyl chloride[20] as alkylating agent and 50°C as reaction temperature. Under these conditions reaction of **7** with benzylchloride, 2-chloropropane, 1-chlorobutane and chloromethylthiomethyl gave **32-35** in 68, 76, 77% and 48% yield, respectively. In the case of the O-methyl derivative **36** we applied a general method[18a] for the preparation of methyloxime ethers -*viz.* reaction of **7** with methyliodide in acetone in the presence of suspended Ag_2O (86% yield). Reduction of the oxime ethers was accomplished under standard conditions. Treatment of **32-36** with borane-trimethylamine complex afforded the target compounds **45-49** in 56-91% yield.

N-Hydroxytryptamine derivatives were not efficiently accessible *via* the oxime approach (*vide supra*) and therefore we explored an alternative approach towards the N-alkoxy derivatives, from the corresponding N-hydroxy compounds. (Route B', Scheme III). The problem we faced was the selective O-alkylation of the N-mono substituted hydroxylamine function. In general N,O-disubstituted hydroxylamines are prepared by O-alkylation of N-hydroxyurethanes followed by acidic hydrolysis.[21] By a careful choice of the protective group this method could be made suitable for

Scheme III



our goal. The protective group not only has to be easily incorporated and removed but also has to survive the alkylation conditions. The trichloroethoxycarbonyl (TrOC) group satisfied the first two criteria but failed with respect of the last one. The protective group of choice which met all of the criteria was the 2-(trimethylsilyl)ethoxycarbonyl (TEOC) group.[22] Treatment of **23**, **28** and **29** with 2-(trimethylsilyl)ethylchloroformate in dichloromethane / dioxane[23] at room temperature gave **37**, **38** and **39** in 96%, 91% and 80%, respectively.

The reaction conditions for the O-alkylation of these TEOC-protected compounds depend on the α-substituent in the tryptamine moiety. A N-acyl-N-hydroxytryptophan derivative such as **37** is sensitive to elimination reactions under basic conditions. In the absence of a nucleophile, rearrangement to the corresponding enamine ester derivative has been reported[3b,4g]. Indeed we found that alkylation attempts of **37** with benzylbromide using DMSO/KOtBU or DME/NaH yielded the dehydro ester **53** almost quantitatively. With K_2CO_3 as the base in DMSO at 45°C the desired **40** was obtained in 67% yield. In an analogous fashion reaction of **37** with methyliodide and 1-bromopropane gave **41** and **42** in 69% and 71% yield, respectively. The TEOC-protective group of **40-42** was removed with tetrabutylammonium fluoride (Bu_4NF) in THF to give the N-alkoxytryptophan

derivatives **45**, **46** and **50** in 84%, 89%, and 91% yield, respectively. Since **38** and **39** are less prone to undergo this elimination reaction these compounds could be smoothly converted into **43** and **44** respectively using methyliodide and NaH in DME at room temperature. These compounds were not purified, but immediately deprotected with Bu_4NF in THF yielding the compounds **51** and **52** in an overall yield of 68% and 70%, respectively.

Secondary alkylhalides like 2-bromopropane failed to undergo alkylation with **37-39**. So by this approach (Route B') compound **47** was not accessible in contrast to the oxime approach (Route A').

Conclusions

An efficient route to N-hydroxy-tryptophan and -tryptamine derivatives has been described *via* the corresponding nitro compounds (**18-22**) prepared from gramine (**13**) and nitromethane derivatives (**14-17**). Reduction of the nitro group with Al-amalgam gives the N-hydroxy compounds **23** and **27-30** in high yields (Scheme II, route B).

N-Alkoxytryptophans can be conveniently prepared by a regioselective O-alkylation of oxime **7** followed by reduction with $Me_3N.BH_3$ (Scheme III, route A'). N-Alkoxy-tryptamines and -tryptophans have been prepared by selective N-protection of hydroxylamines **23**, **28** and **29** with 2-(trimethylsilyl)ethyl chloroformate providing **37-39**, and subsequent O-alkylation and removal of the TEOC-group. (Scheme III, route B').

Experimental Section

Melting points were taken on a Koefler hot stage (Leitz-Wetzlar) and are uncorrected. Ultraviolet spectra were measured with a Perkin Elmer spectrometer, Model lambda 5. Proton magnetic resonance spectra were measured on a Bruker WH-90 or on a Bruker AM 400 spectrometer. Chemical shifts are reported as δ-values relative to tetramethylsilane as an internal standard; deuteriochloroform was used as solvent unless stated otherwise. Mass spectra were obtained with a double-focusing VG 7070E spectrometer. Thin-layer chromatography (TLC) was carried out by using silica gel F-254 plates (thickness 0.25 mm). Spots were visualized with a UV hand lamp, iodine vapor, or Cl_2-TDM.[24] For column chromatography Merck silica gel (type 60H) was used.

Ethyl α-(Hydroxyimino)-β-(4-chloroindol-3-yl)propanate (8). Ethyl α-hydroxyimino--β-bromopropanoate (2.3 g., 11 mmol) in CH_2Cl_2 (40 mL) was added dropwise to a stirred solution of **3** (5.0 g., 33 mmol) and a suspension of Na_2CO_3 (2.4 g., 23 mmol) in CH_2Cl_2 (30 mL) at room temperature under argon. Stirring was continued at room temperature for 22h. The mixture was then filtered through a thin layer silica gel (60) and concentrated to dryness. The residue was subjected to column chromatography (silica gel 60H, EtOAc/n-hexane,1/2,v/v) to yield 6.3 g. of crystalline **8**, 67%. It was recrystallized from CH_2Cl_2/n-hexane: mp 166-171°C; Rf 0.35 (solvent system C); UV (MeOH) λ_{max} 224, 284 nm, λ_{min} 253 nm; EIMS (70 eV) m/z 282 ($[M+2]^+$, 12%)· 280 (M^+, 34%), 265 ($[M+2-OH]^+$, 21%), 263 ($[M-OH]^+$,56%), 192 ($[C_{10}H_7N_2Cl]^+$, 28%), 190 ($[C_{10}H_7N_2Cl]^+$, 81%), 166 ($[C_9H_7NCl]^+$, 33%), 164 ($[C_9H_7NCl]^+$, 100%); ^1H NMR δ 8.76 (br s, 1H, NH), 7.27-6.98 (m, 3H, indole C(5)-C(7)H), 6.84 (s, 1H, indole C(2)H), 4.45 (s, 2H, indole C(3)-CH_2), 4.26 (q, 2H, OCH_2CH_3), 1.23 (t, 3H, OCH_2CH_3); Anal.Calc. for $C_{13}H_{13}ClN_2O_3$ (MW 280.713): C, 55.62; H,4.67; N, 9.98. Found: C, 55.25; H, 4.67; N, 9.91.

Ethyl α-(Hydroxyimino)-β-(5-chloroindol-3-yl)-propanoate (9). Identical procedure with 5-choroindole **4** gave **9**, yield 70%. Recrystallized from CH_2Cl_2/n-hexane: mp 164-166°C; Rf 0.30 (solvent system C); UV (MeOH) λ_{max} 224, 290 nm, λ_{min} 253 nm; EIMS (70 eV) m/z 282 ($[M+2]^+$, 14%)· 280 (M^+, 41%), 265 ($[M+2-OH]^+$, 22%), 263 ($[M-OH]^+$,64%), 192 ($[C_{10}H_7N_2Cl]^+$, 34%), 190 ($[C_{10}H_7N_2Cl]^+$, 77%), 166 ($[C_9H_7NCl]^+$, 34%), 164 ($[C_9H_7NCl]^+$, 100%); ^1H NMR ($CDCl_3$ /

CD_3OD,95/5,v/v) δ 7.70 (d, 1H, indole C(7)H), 7.30-6.98 (m, 3H, indole C(2)H,C(4)H and C(6)H), 4.23 (q, 2H, OCH_2CH_3), 3.98 (s, 2H, indole C(3)-CH_2), 1.27 (t, 3H, OCH_2CH_3); Anal.Calc. for $C_{13}H_{13}ClN_2O_3$ (MW 280.713): C, 55.62; H,4.67; N, 9.98. Found: C, 55.42; H, 4.67; N, 9.95.

3-(2-Hydroxyimino-2-phenylethyl)indole (12)

A solution of α-(hydroxyimino)-α-phenyl-β-bromoethane[9] (21.4 g, 100 mmol) in dry dichloromethane (400 mL) was added dropwise in 4 hours to a solution of indole (**1**) (35.1 g, 300 mmol) and Na_2CO_3 (21.1 g, 200 mmol) in dry dichloromethane (250 mL). Stirring was continued for 40 hours at room temperature in an argon atmosphere. The reaction mixture was filtered and subsequently washed with 0.1 N HCl and brine. The organic layer was dried (Na_2SO_4) and the solvent evaporated in vacuo. The residue was subjected to column chromatography (CH_2Cl_2/MeOH, 98/2, v/v) to give 5 g (20%) of **12**: mp 167-171°C; Rf 0.29 (CH_2Cl_2/MeOH, 98/2, v/v) ; EIMS (70 eV) m/z (relative intensity) 250 (M^+, 48), 233 ($[M\text{-}OH]^+$, 13), 130 ($[C_9H_8N]^+$, 100); ^1H NMR δ 8.22-7.00 (m, 10H, C(4)-C(7)H, indole NH and C_6H_5), 6.89 (m, 1H, C(2)H), 4.27 (s, 2H, indole C(3)-CH_2); Anal.Calcd. for $C_{16}H_{14}N_2O$ (Mw 250.303): C, 76.78; H, 5.64; N, 11.19. Found: C, 76.78; H, 5.60; N, 10.96.

3-(2-Nitro-2-phenylethyl)indole (18)

Following the same procedure as described by Heath-Brown[12] for **20**, with gramine (5.8 g, 30 mmol), sodium (0.76 g, 33 mmol) and dimethylsulfate (7.56 g, 60 mmol) in ethanol (25 mL) and α-nitrotoluene (**15**)[13] (5.1 g, 37 mmol) gave after column chromatography ($CHCl_3$/n-hexane, 70/30, v/v) 5.0 g (63%) of **18**. Recrystallized from CH_2Cl_2/n-hexane: mp 98-100°C; Rf 0.58 ($CHCl_3$); EIMS (70 eV) m/z (relative intensity) 266 (M^+, 29), 220 ($[C_{16}H_{14}N]^+$, 100), 130 ($[C_9H_8N]^+$, 36); ^1H NMR δ 8.00 (br s, 1H, NH), 7.56-7.04 (m, 9H, indole C(4)-C(7)H and C_6H_5), 6.91 (d, 1H, indole C(2)H), 5.77 (X part of ABX spectrum, 1H, J=5.5Hz, J=9.3Hz, CH_2CHNO_2), 3.95 and 3.47 (AN part of ABX spectrum, 2H, ^2J=14.7Hz, J=5.5Hz, J=9.3Hz, CH_2CHNO_2); Anal.Calcd. for $C_{16}H_{14}N_2O_2$ (Mw 266.302): C, 72.16; H, 5.30; N, 10.52. Found: C, 71.80; H, 5.24; N, 10.36.

3-(2-Nitroethyl)indole (19) and 3-[2-Nitro-2(3-indolyl-methyl)ethyl]indole (31)

This synthesis is a modification of Heath-Brown's procedure.[12]

Sodium methoxide which was freshly prepared from 4.35 g (189 mmol) sodium in dry methanol (300 mL) was added to a stirred solution of gramine (**13**) (30 g, 172 mmol) and dimethylsulfate (43.4 g, 344 mmol) in nitromethane/methanol, 1/1, v/v (500 mL). The reaction mixture was stirred for 24h. After the solution had been concentrated to near dryness, the residue was dissolved in dichloromethane and subsequently washed with 5% NH_3 and 1N HCl and brine. The organic layer was dried (Na_2SO_4) en the solvent evaporated in vacuo. The crystalline residue was subjected to column chromatography ($CHCl_3$/n-hexane, 75/25, v/v) to yield 28g (86%) of **19** and 2.8g (5%) of **31**.

Compound 19: Spectrocopic data are identical with earlier published results.[7a,d]

Compound 31: Recrystallized from CH_2Cl_2/MeOH/n-hexane: mp 213-214°C; Rf 0.29 ($CHCl_3$); EIMS (70 eV), m/z (relative intensity) 319 (M^+, 67), 272 ($[M\text{-}HNO_2]^+$, 22), 130 ($[C_9H_8N]^+$, 100); ^1H NMR (DMSO-d^6) δ 10.96 (br s, 2H, 2xNH), 7.60-6.92 (m, 10H, 2x indoleC(2)H and 2xindoleC(4)-C(7)H), 5.22 (X part of ABX spectrum, 1H, $CHNO_2$), 3.50 (AB part of ABX spectrum, 4H, 2x indoleC(3)-CH_2); Anal Calcd. for $C_{19}H_{17}N_3O_2$ (Mw 319.364): C, 71.46; H, 5.37; N, 13.16. Found: C, 71.21; H, 5.32; N, 12.97.

N(1)[(*tert*-Butyloxy)carbonyl]-3-(2-Nitropropyl)indole (21)

To a stirred solution of **20**[12] (204 mg, 1 mmol) and DMAP (12.2 mg, 0.1 mmol) in dry acetonitrile (3 mL) was added di-*tert*-butyloxycarbonate (260 mg, 1.2 mmol). After completion of the reaction as was monitored by TLC ($CHCl_3$/MeOH, 99/1, v/v) the reaction mixture was diluted with ethyl acetate and subsequently washed with 10% NH_3, water and brine. The organic layer was dried ($MgSO_4$) and the solvent evaporated in vacuo. The residue was subjected to column chromatography (CH_2Cl_2/n-hexane, 90/10, v/v) to give 250 mg (83%) of **21**. Recrystallized from CH_2Cl_2/n-hexane; mp 117-119°C; Rf 0.82 ($CHCl_3$); UV (MeOH) λmax 225, 257, 262.5, 273 (sh), 284.5, 292.5 nm; EIMS (70 eV) m/z (relative intensity) 304 (M^+, 34), 248 (16), 201 (38), 157 ($[C_{11}H_{11}N]^+$, 54), 57 ($[C_4H_9]^+$, 100); ^1H NMR δ 8.07 (m, 1H, C(2)H), 7.51-7.12 (m, 4H, C(4)-C(7)H), 4,86 (m, 1H, CH_2CHCH_3), 3.43 and 3.12 (AB part of ABX spectrum, 2H, ^2J=14.7Hz, J=6.3Hz, J=5.4Hz, indole C(3)-CH_2), 1.67 (s, 9H, $C(CH_3)_3$), 1.60 (d, 3H, J=6.3Hz, $CHCH_3$); Anal.Cald. for $C_{16}H_{20}N_2O_4$ (Mw 304.349): C, 63.14; H, 6.62; N, 9.20. Found: C, 63.24; H, 6.59; N, 9.13.

Ethyl α-(Hydroxyamino)-β-(indol-3-yl)propanoate (23)
Procedure A: Reduction of oxime **7** as described earlier.[3]
Procedure B: This synthesis is a modification of Cohen's procedure.[14] To a stirred solution of **22**[15] (524 mg, 2 mmol) in ethyl acetate (saturated with water) (100 mL) was added portionwise at 0°C freshly prepared Al(Hg) untill the starting material was consumed. The reaction mixture was filtered and the filtrate dried ($MgSO_4$) and the solvent evaporated in vacuo. The residue was subjected to column chromatography ($CHCl_3$/MeOH, 98/2, v/v) to give 455 mg (92%) of **23**. Spectroscopic data are identical with earlier published results.[3]

Ethyl α-(Hydroxyamino)-β-(4-chloroindol-3-yl)-propanoate (24). A solution of HCl in ethanol (13 mL of a 7N solution) was added dropwise to a stirred solution of **8** (2.0 g., 7.2 mmol) and $(CH_3)_3N.BH_3$ (Aldrich Chemical Co; 590 mg, 8.1 mmol) in EtOH (25 mL) at room temperature and in argon atmosphere. Stirring was continued for 2.5 h. The mixture was then concentrated to near dryness. The residue dissolved in CH_2Cl_2. This solution was neutralized with $NaHCO_3$ and filtered. The filtrate was washed with 0.1 N HCl and dried over Na_2SO_4. Evaporation of the solvent in vacuo and recrystallization of the residue from CH_2Cl_2/MeOH/n-hexane gave 1.48 g. **24** (73%): mp 138-140°C; Rf 0.25 (solvent system D); UV (MeOH) λ_{max} 224, 289 nm, λ_{min} 251 nm; EIMS (70 eV) m/z 284 ($[M+2]^+$, 0.5%)· 282 (M^+, 1.7%), 166 ($[C_9H_7NCl]^+$, 34%), 164 ($[C_9H_7NCl]^+$, 100%); ^1H NMR ($CDCl_3/CD_3OD$,95/5,v/v) δ 7.35-7.00 (m, 4H, indole C(2)H,C(5)H- C(7)H), 4.17 (q, 2H,^3J 7.1 Hz, OCH_2CH_3), 4.01 (X part of ABX spectrum, 1H,^3J 5.3 Hz, ^3J 8.9 Hz, indole C(3)-CH_2-CH),3.40 and 3.23 (AB part of ABX spectrum, 2H,^2J 14.2 Hz, ^3J 5.3 Hz, ^3J 8.9 Hz, indole C(3)-CH_2), 1.19 (t, 3H, OCH_2CH_3).

Ethyl α-(Hydroxyamino)-β-(5-chloroindol-3-yl)-propanoate (25). Identical procedure with **9** gave **25**, yield 94%. Recrystallized from CH_2Cl_2/MeOH/n-hexane: mp 188-190°C; Rf 0.26 (solvent system D); UV (MeOH) λ_{max} 224, 289 nm, λ_{min} 251 nm; EIMS (70 eV) m/z 284 ($[M+2]^+$, 1.3%)· 282 (M^+, 4.7%), 166 ($[C_9H_7NCl]^+$, 31%), 164 ($[C_9H_7NCl]^+$, 100%); ^1H NMR ($CDCl_3/CD_3OD$,95/5,v/v) δ 11.34 (br s, 1H, NH), 7.61-7.04 (m, 4H, indole C(2)H,C(4)H and C(6)-C(7)H), 4.28 (X part of ABX spectrum, 1H,^3J 3.9 Hz, ^3J 9.6 Hz, indole C(3)-CH_2-CH), 4.07 (q, 2H,^3J=7.1 Hz, OCH_2CH_3), 3.46 and 3.23 (AB part of ABX spectrum, 2H,^2J 14.4 Hz, ^3J 3.9 Hz, ^3J 9.6 Hz, indole C(3)-CH_2), 0.97 (t, 3H, ^3J=7.1 Hz, OCH_2CH_3).

3-(2-Hydroxyamino-2-phenylethyl)indole (27)
Procedure A: To a solution of **12** (1.63 g, 6.5 mmol) in dry methanol (150 mL) was added 5 mL of a solution of $NaCN.BH_3$ (4 g, 63.7 mmol in 50 mL dry methanol). By adding slowly a solution of HCl in ethanol (7N) the mixture was kept at pH 1~2 (electronic pH-meter) untill the pH did not change anymore. Another 5mL of the $NaCNBH_3$ solution was than added and again the pH was adjusted to pH 1~2 with the ethanolic HCl solution. This process was repeated untill the total $NaCN.BH_3$ soltution (50 mL) was added (48 hours). The reaction mixture was filtered and the residue concentrated to dryness. The residue was dissolved in ethylacetate/water, 1/1, v/v. The aqueous layer was washed again with ethyl acetate. The combined organic layers were subsequently washed with a saturated $NaHCO_3$ solution and brine. The organic layer was dried ($MgSO_4$) and the solvent evaporated in vacuo. Recrystallization from CH_2Cl_2/n-hexane gave 1.6 g (95%) of **27**.: mp 112-115°C; Rf 0.75 (EtOAc/n-hexane, 9/1, v/v); ^1H NMR δ 7.93 (br s, 1H, NH), 7.71-6.95 (m, 9H,indole C(4)-C(7)H and C_6H_5), 6.82 (d, 1H, C(2)H), 4.64 (br s, 2H, HNOH), 4.27 (X part of ABX spectrum, 1H, J=14.1Hz, indole C(3)CH_2CH), 3.17 and 3.05 (AB part of ABX spectrum, 2H, ^2J=14.4Hz, J=6.0Hz, J=8.6Hz, indole C(3)CH_2CH); Anal.Calcd. for $C_{16}H_{16}N_2O$ (Mw 252.319): C, 76.16; H, 6.39: N, 11.10. Found: C, 76.18; H, 6.42; N, 10.86.
Procedure B: : The same procedure was followed as was described for **23**. Reaction of **18** (2.14 g, 8 mmol) with Al(Hg) gave after column chromatography ($CHCl_3$/MeOH, 98/2, v/v) 1.54 g (76%) of **27**.

3-(2-Hydroxyaminoethyl)indole (28)
Procedure B was followed as described for **23**. Reaction with **19** (1.5 g, 7.9 mmol) gave after evaporation of the solvent crude **28**. The residue was not purified because of the instability of the compound. Spectroscopic data are identical with earlier published results.[7]

3-(2-Hydroxyaminopropyl)indole (29)
The same procedure was followed as described for **23**. Reaction with **20** (8,23 g, 40.3 mmol) gave after column chromatography ($CHCl_3$/MeOH, 96/4, v/v) 7.5 g (98%) of **29**. Spectroscopic data are identical with earlier published results.[12b]

N(1)-[(*tert*-Butyloxy)carbonyl]-3-(2-Hydroxyaminopropyl)indole (24)
The same procedure was followed as described for **23**. reaction with **21** (145 mg, 0.48 mmol) gave after column chromatography ($CHCl_3$/MeOH, 99/1, v/v) 74 mg (54%) of **30**. Rf 0.20 ($CHCl_3$/MeOH, 97/3, v/v); EIMS (70 eV) m/z (relative intensity) 290 (M^+, 21), 189 (38), 130 ($[C_9H_8N]^+$,100); ^1H NMR δ 8.17 (m, 1H, C(2)H), 7.67-7.19 (m, 4H, C(4)-C(7)H), 6.21 (br s, 2H, HNOH), 3.31 (m, 1H, CH_2CHCH_3), 2.85 (t, 2H, indole C(3)-CH_2), 1.68 (s, 9H, $C(CH_3)_3$), 1.15 (d, 3H, $CHCH_3$).

Ethyl α-(2-propyloximino)-β-(indol-3-yl)propanoate (33)
To a stirred solution of **7**[3,4] (375 mg, 1.52 mmol) and 2-chloropropane (235 mg, 3 mmol) in DMSO (7 ML) at 50°C was added portionwise KOtBu (188 mg, 1.68 mmol). After completion of the reaction (3 hours) as was monitored by TLC ($CHCl_3$/MeOH, 97/3, v/v) the reaction mixture was diluted with dichloromethane (50 mL) and successively washed with water (3x) and brine. The organic layer was dried (Na_2SO_4) and the solvent evaporated in vacuo. The residue was subjected to column chromatography ($CHCl_3$) to give 330 mg (76%) of **33**. Oil; Rf 0.37 ($CHCl_3$/MeOH, 97/3, v/v); EIMS (70 eV) m/z (relative intensity) 288 (M^+, 32), 229 ($[M\text{-}OC_3H_7]^+$, 51), 155 (100), 130 ($[C_9H_8N]^+$, 93); ^1H NMR δ 8.12 (br s, 1H, NH), 7.90-7.79 (m, 1H, indole (4)H), 7.34-7.06 (m, 4H, indole C(2)H and C(5)-C(7)H), 4.68 (m, 1H, $NOCH(CH_3)_2$), 4.27 (q, 2H, OCH_2CH_3), 4.07 (s, 2H, indole C(3)CH_2), 1.40 (d, 6H, $NOCH(CH_3)_2$), 1.29 (t, 3H, OCH_2CH_3).

Ethyl α-(1-butyloximino)-β-(indol-3-yl)propanoate (34)
The same procedure was followed as described for **33**. Reaction with **7**[3,4] (984 mg, 4 mmol), 1-chlorobutane (1.2 g, 12 mmol) and KOtBu (0.5 g, 4.4 mmol) in DMSO at 50°C gave after column chromatography ($CHCl_3$) 930 mg (77%) of **34**. Oil; Rf 0.83 ($CHCl_3$/MeOH, 97/3, v/v); EIMS (70 eV) m/z (relative intensity) 302 (M^+, 30), 229 ($[M\text{-}COOEt]^+$, 60), 155 (100), 130 ($[C_9H_8N]^+$, 80); ^1H NMR δ 8.09 (br s, 1H, indole NH), 7.76-7.03 (m, 5H, C(2)H and C(4)-C(7)H), 4.30 (t, 1H, $NOCH_2$),4.21 (q, 2H, OCH_2CH_3), 4.02 (d, 2H, indole C(3)CH_2), 1.88-1.13 (m, 4H, $OCH_2CH_2CH_2CH_3$), 1.24 (t, 3H, OCH_2CH_3), 0.92 (t, 3H, $OCH_2CH_2CH_2CH_3$).

Ethyl α-(methylthiomethyloximino)-β-(indol-3-yl)propanoate (35)
The same procedure was followed as described for **33**. Reaction with **7**[3,4] (984 mg, 4 mmol), α-chloromethylmethylsulfide (580 mg, 6 mmol) and KOtBu (493 mg, 4.4 mmol) in DMSO at room temperature gave after column chromatography ($CHCl_3$) 586 mg (48%) of **35**. Oil; Rf 0.80 ($CHCl_3$/MeOH, 97/3, v/v); EIMS (70 eV) m/z (relative intensity) 306 (M^+, 43), 259 ($[C_{14}H_{15}N_2O_3]^+$, 15), 130 ($[C_9H_8N]^+$, 100); ^1H NMR δ 7.98 (br s, 1H, NH), 7.79-7.02 (m, 5H, indole C(2)H and C(4)-C(7)H), 5.34 (s, 2H, OCH_2S), 4.24 (q, 2H, OCH_2CH_3), 4.07 (s, 2H, C(3)-CH_2), 2.21 (s, 3H, SCH_3), 1.27 (t, 3H, OCH_2CH_3).

Ethyl α-(methyloximino)-β-(indol-3-yl)propanoate (36)
To a stirred solution of **7**[3,4] (246 mg, 1 mmol) and methyliodide (1 mL) in aceton (3 mL) was added Ag_2O (250 mg, 1.08 mmol). After completion of the reaction (30 minutes) as was monitored by TLC ($CHCl_3$/MeOH, 97/3, v/v) the reaction mixture was filtered and the filtrate evaporated in vacuo. The residue was subjected to column chromatography ($CHCl_3$) to give 224 mg (86%) of **36**. Oil; Rf 0.67 ($CHCl_3$/MeOH, 97/3, v/v); EIMS (70 eV) m/z (relative intensity) 260 (M^+, 46), 229 ($[C_{13}H_{13}N_2O_2]^+$, 55), 155 ($[C_{10}H_7N_2]^+$, 100), 130 ($[C_9H_8N]^+$, 100); ^1H NMR δ 8.03 (br s, 1H, NH), 7.69-7.00 (m, 5H, C(2)H and C(4)-C(7)H), 4.25 (q, 2H, OCH_2CH_3), 4.13 (s, 3H, OCH_3), 4.04 (s, 2H, C(3)-CH_2), 1.31 (t, 3H, OCH_2CH_3).

Ethyl α-[N,N-(2-(trimethylsilyl)ethyloxycarbonyl, hydroxy)amino]-β-(indol-3-yl)propanoate (37)
2-(Trimethylsilyl)ethylchloroformate[22] (1.08 g, 6 mmol) was added dropwise to a stirred solution of **23**[3,4] (1.0 g, 4 mmol) in CH_2Cl_2/dioxane, 1/1, v/v (25 mL). The reaction was monitored by TLC. Stirring was continued for 2 hours. The reaction mixture was concentrated to near dryness, dissolved in CH_2Cl_2 and subsequently washed with saturated $NaHCO_3$ and brine and dried with Na_2SO_4.

Evaporation of the solvent gave a crystalline material which was subjected to column chromatography ($CHCl_3$/n-hexane, 99.5/0.5, v/v) to yield 1.51 g. (96%) of **37**. Crystallization from CH_2Cl_2/n-hexane: mp 101-102.5 °C; Rf 0.45 ($CHCl_3$/MeOH, 93/7, v/v); UV (MeOH) λmax 224, 274(sh), 281, 289 nm; CIMS (100 eV) m/z (relative intensity) 393 ($[M+1]^+$, 9), 392 (M^+, 23), 365 (28), 349 (49), 321 (27), 247 ($[M-C_6H_{13}O_2Si]^+$, 11), 231 (53), 216 (67), 215 (95), 130 ($[C_9H_8N]^+$, 100); ^{1}H NMR δ 8.23 (br s, 1H, NH), 7.77-7.19 (m, 5H, indole C(2) and C(4)-C(7)H), 6.53 (br s, 1H, NOH), 5.11 (t, 1H, J=7.8 Hz, HCCOOEt), 4.37 (q, 2H, OCH_2CH_3), 3.91 (m, 2H, OCH_2CH_2Si), 3.51 (d, 2H, J=7.8 Hz, Indole C(3)CH_2), 1.39 (t, 3H, OCH_2CH_3), 0.71 (m, 2H, CH_2Si), 0.0 (s, 9H, $Si(CH_3)_3$); Anal.Calcd. for $C_{19}H_{28}N_2O_5Si$ (Mw 392.531): C, 58.14; H, 7.19; N, 7.14. Found: C, 57.83; H, 7.14; N, 7.16.

3-[2-(N,N-(2-(Trimethylsilyl)ethyloxycarbonyl, hydroxy)amino)ethyl]indole (38)

The same procedure was followed as described for **37**. 2-(Trimethylsilyl)ethylchloroformate[22] (675 mg, 3.75 mmol) and **28** (440 mg, 2,5 mmol) gave after column chromatography (EtOAc/n-hexane, 40/60, v/v) 750 mg (91%) of **38**. Crystallization from EtOAc/n-hexane: mp 95-97°C; Rf 0.39 ($CHCl_3$/MEOH, 97/3, v/v)); UV (MeOH) λmax 224, 274(sh), 281, 289 nm; EIMS (70 eV) m/z (relative intensity) 320 (M^+, 1), 157 ($[C_{10}H_9N_2]^+$, 11), 130 ($[C_9H_8N]^+$, 100); ^{1}H NMR δ 8.02 (br s, 1H, NH), 7.70-7.04 (m, 5H, indole C(2) and C(4)-C(7)H), 6.25 (br s, 1H, NOH), 4.11-3.87 (m, 2H, OCH_2CH_2Si), 3.90 (t, 2H, CH_2N), 3.13 (t, 2H, Indole C(3)CH_2), 0.89-0.68 (m, 2H, CH_2Si), 0.0 (s, 9H, $Si(CH_3)_3$); Anal.Calcd. for $C_{16}H_{24}N_2O_3Si$ (Mw 320469): C, 59.97; H, 7.55; N, 8.74. Found: C,59.91; H,7.62; N, 8.70.

3-[2-(N,N-(2-(Trimethylsilyl)ethyloxycarbonyl, hydroxy)amino)propyl]indole (39)

The same procedure was followed as described for **37**. 2-(Trimethylsilyl)ethylchloroformate[22] (1625 mg, 9 mmol) and **29** (1.14 g, 6 mmol) gave after column chromatography ($CHCl_3$/n-hexane, 99/1, v/v) 1.61 g (80%) of **39**. Crystallization from $CHCl_3$/n-hexane:mp 122-125 °C; Rf 0.29 ($CHCl_3$/MeOH, 97/3, v/v); UV (MeOH) λmax 224, 274(sh), 281, 289 nm; CIMS (100 eV) m/z (relative intensity) 334 (M^+, 2), 291 (14), 230 (9), 158 ($[C_{11}H_{12}N]^+$, 100), 130 ($[C_9H_8N]^+$, 85), 73 ($[Si(CH_3)_3]^+$, 100); ^{1}H NMR δ 8.07 (br s, 1H, NH), 7.68-7.07 (m, 5H, indole C(2) and C(4)-C(7)H), 6.20 (br s, 1H, NOH), 4.53 (m, 1H, $HCCH_3$), 3.84 (m, 2H, OCH_2CH_2Si), 3.18 and 2.94 (AB part of ABX spectrum, 2H, ^{2}J=14.3 Hz, J=8.1 Hz, J=6.0 Hz, indole C(3)CH_2), 1.36 (d, 3H, J=7.0Hz, $HCCH_3$), 0.63 (m, 2H, CH_2Si), 0.0 (s, 9H, $Si(CH_3)_3$); Anal.Calcd. for $C_{17}H_{26}N_2O_3Si$ (Mw 334.494): C, 61.04; H, 7.83; N, 8.37. Found: C, 60.83; H, 7.93; N, 8.16.

Ethyl α-(benzyloxamino)-β-(indol-3-yl)propanoate (45)

Procedure A: A solution of HCl in ethanol (5 mL of a 7N solution) was added to a stirred solution of **32**[3] (672 mg, 2.0 mmol) and borane-trimethylamine complex (TMA.BH_3:Aldrich Chemical Co., 210 mg, 2.9 mmol) in ethanol (5 mL) at room temperature. Stirring was continued for 24h at room temperature. The reaction mixture was then concentrated to dryness in vacuo. The residue was dissolved in dichloromethane and subsequently washed with saturated $NaHCO_3$, water and brine. The organic layer was dried (Na_2SO_4) and the solvent evaporated in vacuo. The residue was subjected to column chromatography ($CHCl_3$/MeOH, 98/2, v/) to give 566 mg (84%) of **45**. Spectroscopic data are identical with results published earlier.[3]

Procedure B: K_2CO_3 (168 mg, 1.22 mmol) was added portionwise to a stirred solution of **37** (480 mg, 1.22 mmol), benzylbromide (415 mg, 2.44 mmol) in DMSO (12 mL). After completion of the reaction (2 days) as was monitored by TLC ($CHCl_3$/MeOH, 99/1, v/v) the reaction mixture was diluted with dichloromethane (50 mL) and washed with water and brine. The organic layer was dried (Na_2SO_4) and the solvent evaporated in vacuo. The residue was subjected to column chromatography ($CHCl_3$/MeOH, 98/2, v/v) to give 394 mg (67%) of **40**. The product was immediately deprotected by addition of tetrabutylammoniumfluoride (TBAF) (1.7 mL of a 1N solution on THF) to a stirred solution of **40** in dry THF (5 mL). Stirring was continued for 5h after which the reaction mixture was washed with water and brine. The organic layer was dried ($MgSO_4$) and the solvent evapoarted in vacuo. The residue was subjected to column chromatography (EtOAc/n-hexane, 25/75, v/v) to give 232 mg (84%) of **45**.

Ethyl α-(methyloxamino)-β-(indol-3-yl)propanoate (46)

Procedure A: The same procedure was followed as described for **45**. Reaction with **36** (624 mg, 2.4 mmol) and TMA.BH_3 (210 mg, 2.9 mmol) gave after column chromatography ($CHCl_3$/MeOH,

98/2, v/) 456 mg (73%) of **46**. Oil; Rf 0.20 ($CHCl_3$/MeOH, 97/3, v/v); EIMS (70 eV) m/z (relative intensity) 262 (M^+, 41), 189 ($[C_{11}H_{13}N_2O]^+$, 18), 130 ($[C_9H_8N]^+$, 100); ^{1}H NMR δ 8.12 (br s, 1H, indole NH), 7.66-7.07 (m, 5H, C(2)H and C(4)-C(7)H), 4.78 (br s, 1H, HNOMe), 4.11 (q, 2H, OCH_2CH_3), 4.02 (t, 1H, CHCOOEt), 3.49 (s, 3H, OCH_3), 3.09 (d, 2H, C(3)CH_2CH), 1.14 (t, 3H, OCH_2CH_3).

Procedure B: The same procedure was followed as described for **45**. With **37** (392 mg, 1 mmol), MeI (156 mg, 1.1 mmol) and K_2CO_3 (138 mg, 1 mmol) in DMSO gave after column chromatography ($CHCl_3$) 280 mg (69%) of **41.** Deprotection with Bu_4NF gave 161 mg (89%) of **46**.

Ethyl α-(2-propyloxamino)-β-(indol-3-yl)propanoate (47)

Procedure A was follwoed as described for **45**. Reaction with **33** (1008 mg, 3.5 mmol) and TMA.BH_3 (281 mg, 3.85 mmol) gave after column chtomatography ($CHCl_3$/MeOH, 99/1, v/v) 673 mg (74%) of **47**. Oil; Rf 0.27 ($CHCl_3$/MeOH, 99/1, v/v); EIMS (70 eV) m/z (relative intensity) 290 (M^+, 53), 217 ($[C_{13}H_{17}N_2O]^+$, 22), 130 ($[C_9H_8N]^+$, 100); ^{1}H NMR δ 8.03 (br s, 1H, indole NH), 7.66-7.02 (m, 5H, C(2)H and C(4)-C(7)H), 4.11 (q, 2H, OCH_2CH_3), 4.03-3.3.74 (m, 2H, $OCH(Me)_2$ and CHCOOEt), 3.09 (d, 2H, C(3)CH_2CH), 1.14 (t, 3H, OCH_2CH_3), 1.10 (d, 6H, $CH(CH_3)_2$).

Ethyl α-(1-butyloxamino)-β-(indol-3-yl)propanoate (48)

Procedure A was followed as described for **45**. Reaction with **34** (900 mg, 3.0 mmol) and TMA.BH_3 (460 mg, 6.3 mmol) gave after column chtomatography ($CHCl_3$) 833 mg (91%) of **48**. Oil; Rf 0.35 ($CHCl_3$/MeOH, 99/1, v/v); EIMS (70 EV) m/z (relative intensity) 304 (M^+, 52), 231 ($[M\text{-}COOEt]^+$, 24), 130 ($[C_9H_8N]^+$, 100); ^{1}H NMR δ 8.06 (br s, 1H, indole NH), 7.67-7.04 (m, 5H, C(2)H and C(4)-C(7)H), 4.14 (q, 2H, OCH_2CH_3), 4.01 (t, 1H, CHCOOEt), 3.70 (t, 1H, $NOCH_2$), 3.10 (d, 2H, C(3)CH_2CH), 1.62-1.13 (m, 4H, $OCH_2CH_2CH_2CH_3$), 1.13 (t, 3H, OCH_2CH_3), 0.89 (t, 3H, $OCH_2CH_2CH_2CH_3$).

Ethyl α-(methylthiomethyloxamino)-β-(indol-3-yl)propanoate (49)

Procedure A was followed as described for **45**. Reaction with **35** (520 mg, 1.7 mmol) and TMA.BH_3 (620 mg, 8.5 mmol) gave after column chtomatography ($CHCl_3$) 377 mg (72%) of **49**. Oil; Rf 0.24 ($CHCl_3$/MeOH, 99/1, v/v); EIMS (70 eV) m/z (relative intensity) 308 (M^+, 15), 190 (22), 130 ($[C_9H_8N]^+$, 100); ^{1}H NMR δ 8.03 (br s, 1H, indole NH), 7.62-7.00 (m, 5H, C(2)H and C(4)-C(7)H), 4.77 (s, 2H, OCH_2S), 4.11 (q, 2H, OCH_2CH_3), 4.08 (t, 1H, CHCOOEt), 3.13 (d, 2H, C(3)CH_2CH), 1.14 (t, 3H, OCH_2CH_3).

Ethyl α-(1-propyloxamino)-β-(indol-3-yl)propanoate (50)

Procedure B was followed as described for **45**. Reaction with **37** (480 mg, 1.22 mmol), 1-bromopropane (300 mg, 2.44 mmol) and K_2CO_3 (168 mg, 1.22 mmol) in DMSO (12 mL) gave after column chromatography ($CHCl_3$) 376 mg (71%) of **42**. Oil; Rf0.48 ($CHCl_3$/MeOH, 99/1, v/v); EIMS (70 eV) m/z (relative intensity) 434 (M^+, 4), 215 (40), 130 ($[C_9H_8N]^+$, 100); ^{1}H NMR δ 8.06 (br s, 1H, indole NH), 7.72-7.12 (m, 5H, C(2)H and C(4)-C(7)H), 5.01 (X part of ABX spectrum, 1H, CHCOOEt), 4.27 (q, 2H, OCH_2CH_3), 4.12-3.92 (m, 2H, OCH_2CH_2Si), 3.82 (t, 1H, $NOCH_2$), 3.46 (m, 2H, C(3)CH_2CH), 1.62 (m, 2H, $OCH_2CH_2CH_3$), 1.33 (t, 3H, OCH_2CH_3), 0.92 (t, 3H, $OCH_2CH_2CH_3$), 0.93-0.64 (m, 2H, CH_2Si), 0.00 (s, 9H, $Si(CH_3)_3$). Deprotection with Bu_4NF (1.7 mL of a 1N solution on THF) gave 225 mg (91%) of **50**. Oil; Rf 0.73 ($CHCl_3$/MeOH, 97/3, v/v); EIMS (70 eV) m/z (relative intensity) 290 (M^+, 32), 217 ($[C_{13}H_{17}N_2O]^+$, 20), 130 ($[C_9H_8N]^+$, 100); ^{1}H NMR δ 8.06 (br s, 1H, indole NH), 7.69-7.08 (m, 5H, C(2)H and C(4)-C(7)H), 5.85 (br s, 1H, HNO), 4.14 (q, 2H, OCH_2CH_3), 4.02 (t, 1H, CHCOOEt), 3.67 (t, 1H, $NOCH_2$), 3.10 (d, 2H, C(3)CH_2CH), 1.54 (m, 2H, $OCH_2CH_2CH_3$), 1.17 (t, 3H, OCH_2CH_3), 0.89 (t, 3H, $OCH_2CH_2CH_3$).

3-(2-Methyloxaminoethyl)indole (51)

Sodium hydride (58 mg, 2.42 mmol) was added to a a cooled (-10°C) stirring solution of **38** (700 mg, 2.2 mmol) in dry DME (10 mL) in an argon atmosphere. The reaction mixture was allowed to warm to room temperature by which H_2-evolution occurred. Methyliodide (625 mg, 4.4 mmol) was added to the resulting clear solution. The alkylated product **43** was not isolated but after overnight stirring Bu_4NF (4.4 mL, 1N solution in THF) was added. The reaction mixture was stirred for an additional 8 hours, after which the reaction mixture was diluted with EtOAc (25 mL) and subsequently washed with a saturated $NaHCO_3$ and a 1N HCl and brine solution. The organic layer was dried ($MgSO_4$) and the solvent evaporated in vacuo. The residue was subjected to column

chromatography (EtOAc/n-hexane, 25/75, v/v) 305 mg (68%) of **51**. Oil; Rf 0.38 ($CHCl_3$/MeOH, 97/3, v/v); EIMS (70 eV) m/z (relative intensity) 204 (M^+, 4), 131 (100), 130 ($[C_9H_8N]^+$, 59); ^1H NMR δ 8.03 (br s, 1H, indole NH), 7.67-7.03 (m, 5H, C(2)H and C(4)-C(7)H), 3.56 (s, 3H, $NOCH_3$), 3.48-3.20 (m, 1H, $CHCH_3$), 2.89 (t, 2H, indole C(3)CH_2), 1.13 (d, 3H, CH_3).

3-(2-Methyloxaminopropyl)indole (52)

Sodium hydride (66 mg, 2.75 mmol) was added to a a cooled (-10°C) stirring solution of **39** (835 mg, 2.5 mmol) in dry DME (10 mL) in an argon atmosphere. The reaction mixture was allowed to warm to room temperature by which H_2-evolution occurred. Methyliodide (625 mg, 4.4 mmol) was added to the resulting clear solution. The alkylated product **44** was not isolated but after overnight stirring Bu_4NF (5 mL, 1N solution in THF) was added. The reaction mixture was stirred for an additional 8 hours, after which the reaction mixture was diluted with EtOAc (25 mL) and subsequently washed with a saturated $NaHCO_3$ and a 1N HCl and brine solution. The organic layer was dried ($MgSO_4$) and the solvent evaporated in vacuo. The residue was subjected to column chromatography ($CHCl_3$/n-hexane, 60/40, v/v) to give 330 mg (70%) of **52**. Oil; Rf 0.34 ($CHCl_3$/MeOH, 97/3, v/v); EIMS (70 eV) m/z (relative intensity) 190 (M^+, 20), 130 ($[C_9H_8N]^+$, 100); ^1H NMR δ 8.00 (br s, 1H, indole NH), 7.68-7.05 (m, 5H, C(2)H and C(4)-C(7)H), 3.57 (s, 3H, $NOCH_3$), 3.36-2.93 (m, 4H, indole C(3)CH_2CH_2), 1.78 (br s, 1H, HNO).

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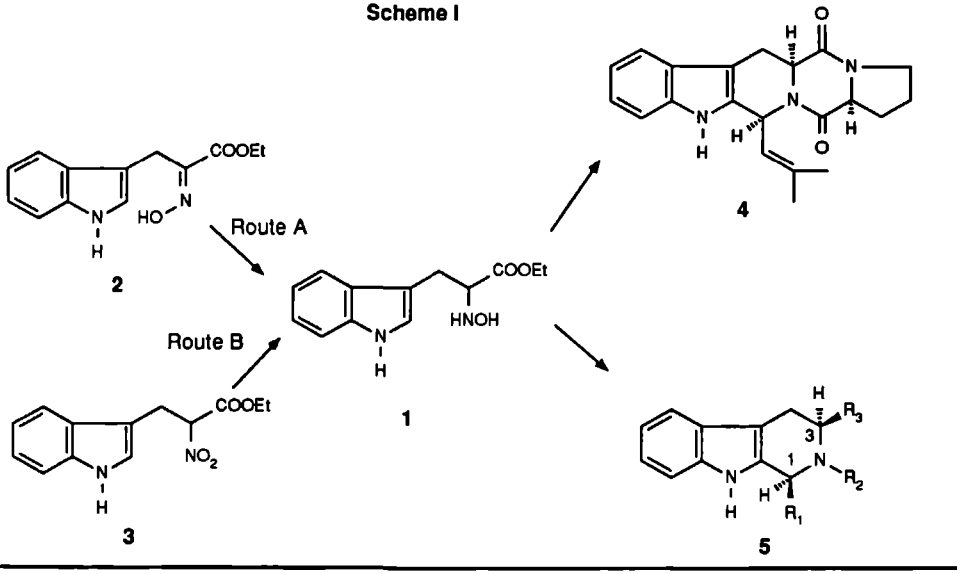
Chapter 2.2

AN APPROACH TO OPTICALLY ACTIVE N-HYDROXYTRYPTOPHAN BY ASYMMETRIC REDUCTION OF THE OXIME DOUBLE BOND.

Although there is strong evidence for a role of N-hydroxytryptophan (**1**) as intermediate in the biosynthesis of several natural products[1], this N-hydroxy-amino acid has still not been obtained in the optically pure form neither by synthesis nor by isolation. It has been demonstrated that **1** can be employed as a building block for chiral natural products such as **4**[2] and in general for 1,3-disubstituted 1,2,3,4-tetrahydro-β-carbolines[3] (*e.g.* **5**). In order to obtain a complete

Scheme I



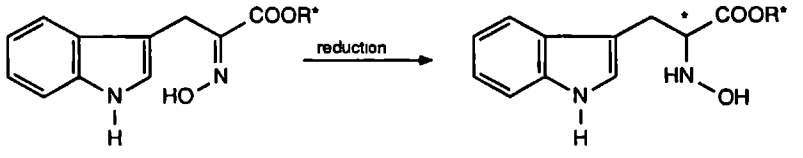
stereoselective total synthesis of these compounds it is most advantageous to start with optically active N-hydroxytryptophan.

So far, Feenstra *et.al.* developed three different approaches to homochiral N-hydroxy-α-amino acid derivatives. The first one features a substitution reaction involving triflates of α-hydroxy esters and hydroxylamine or derivatives thereof.[4] The second approach is based on the selective N-oxidation of derivatives of optically active amino acids[5] and the third one is based on the enzymatic resolution of N-benzyloxy-amino acid ethyl esters.[6] These approaches failed however when tryptophan was involved, mainly due to the reactivity of the indole nucleus.

Consequently an alternative approach was searched for, employing one of the two routes to N-hydroxytryptophan described in Chapter 2.1 (Scheme I, Routes A and B).[7] We reasoned that an

asymmetric reduction of the oxime double bond in Route A might be an attractive avenue to optically active N-hydroxytryptophan. The chirality can be transferred either from a chiral auxiliary group present in the oxime derivative or from a chiral reducing agent. We chose to study the first alternative and for obvious reasons the ester group was considered as a suitable function to introduce chirality. (Scheme II). As chiral auxiliaries the alcohols (-)-menthol, (-)-8-phenylmenthol and

Scheme II



6: R*=(-)-menthyl
7: R*=(-)-8-phenylmenthyl
8: R*=(-)-8-(β-naphtyl)menthyl

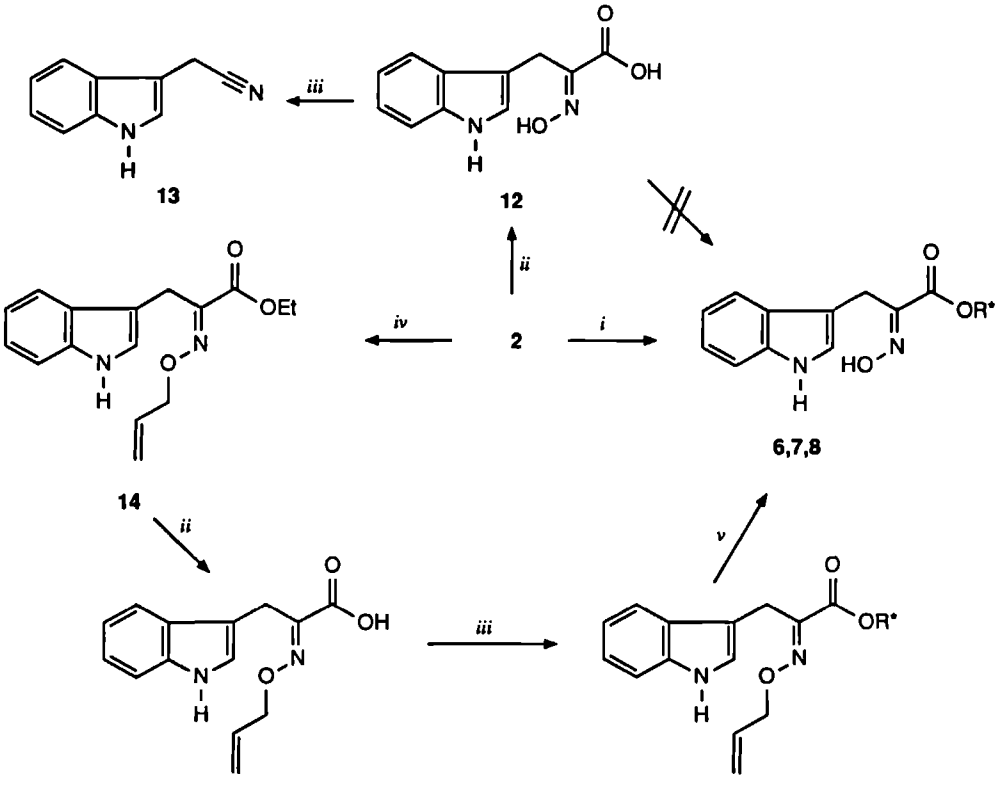
9: R*=(-)-menthyl
10: R*=(-)-8-phenylmenthyl
11: R*=(-)-8-(β-naphtyl)menthyl

(-)-8-(β-naphtyl)menthol were examined. The latter two chiral inductors originally prepared by Corey and Ensley[8] are known to be remarkably efficient, giving excellent asymmetric induction in several types of reactions.[9]

Results

Transesterfication of oxime **2** by a mild method[10] using titanium (IV) isopropoxide and an excess of (-)-menthol (5 equiv.) in dioxane at 85°C gave **6** in 85% yield (Scheme III). This method failed however when the more bulky alcohols 8-phenyl- and 8-(β-naphtyl)menthol were used. Therefore, the direct coupling between the alcohol and an activated carboxylic acid derivative was studied. Saponification of **2** with 2N NaOH in dioxane gave the carboxylic acid **12** (Scheme III) in 70% yield. Activation of the latter with dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP) in the presence of (-)-menthol failed to give the coupling product **6**, but instead 3-indolylacetonitrile (**13**) was obtained. We reasoned that decomposition of **12** in the activation step could be circumvented by the protection of the oxime function. Tsuji[11] introduced the O-allyl ether as a convenient protective group for oximes, which can be deprotected easily by the palladium-catalyzed reaction with triethylammonium formate. We obtained the O-allyl ether **14** in 85% yield by treatment of **2** with KO*t*Bu and allylbromide in DMSO. Saponification of **14** under standard conditions gave the carboxylic acid **15** in 96% yield. Activation of the latter with DCC and DMAP in the presence of (-)-menthol, (-)-8-phenylmenthol or (-)-8-(β-naphtyl)menthol in acetonitrile gave the coupling products **16**, **17** and **18** in yields of 90, 77 or 78%, respectively. The deprotection of the allylic ethers was carried out in refluxing aqueous acetonitrile in the presence of $Pd(OAc)_2$ (0.01 equiv.), triphenylphosphine (0.04 equiv.) and triethylammonium formate (3 equiv.)

Scheme III'



i) $Ti(O\text{-}i\text{-}C_3H_7)_4$/ (-)-menthol, dioxane 85°C, ii) 2N NaOH/dioxane iii) DCC/DMAP / R^*OH iv) allyl bromide/KOtBu, DMSO v) $Pd(OAc)_2$ / PPh_3 / $HCOONHEt_3$, 80% aq.CH_3CN, reflux

to give **6**, **7** and **8** in 89, 96 and 100% yield, respectively.

Reduction of the oxime double bond was accomplished by treatment of **6-8** with trimethylamine-borane complex in dioxane in the presence of hydrochloric acid to give **9-11** (Scheme II). The chemical yields and diastereomeric excesses (de) of the reduction are given in Table I. Reduction of the menthyl derivative **6** (entry 1) gave no induction and a 1/1 mixture of the

Table I

entry	R^*	chem.yield[a]	de(%)[a]
1	(-)-menthyl	85	0
2	(-)-8-phenylmenthyl	72	49
3	(-)-8-(β-naphtyl)menthyl	89	69

a) Based on isolated compounds

diastereomers of **9** were isolated[12]; they could not be separated. Although a significant improvement in the level of induction was obtained with 8-phenylmenthyl ester (entry 2), the de is still moderate. We observed a further increase in the induction level by using (-)-8-(β-naphtyl)menthyl (entry 3). However, a complete diastereofacial control could not be achieved. The two diastereomers of the mixtures **10** and **11** could be readily separated by column chromatography.

The stereochemical outcome can be rationalized with the "π-stacking" model previously proposed by Oppolzer[14]. The aryl group of the inductor shields one face of the oxime double bond, thereby directing the addition of the hydride to the other face. This interpretation is strongly supported by a comparative ^{1}H NMR study that indicates that the shielding of the indole C(3)-CH_2 protons of **6** is identical to that observed for **2**, while these protons in the 8-phenyl- and the 8-(β-naphtyl)menthyl oximes **7** and **8** gave separated signals. In the latter two compounds these diastereotopic protons are shifted upfield (0.5-0.6 ppm (Ha) and 0.1-0.2 ppm (Hb) for **7** and 0.9-1.0 ppm (Ha) and 0.5-0.6 ppm (Hb) for **8** (Chart I). These observations are a firm indication that the "π-stacked" conformation, as

Chart I

	^{1}H NMR δ (ppm)	
	Ha	**Hb**
2	4.20	4.20
6	4.09	4.09
7	3.57	3.97
8	3.22	3.60

depicted in Chart I, exists in solution. It is reasonable to assume that protonation of the oxime nitrogen prior to the attack of the hydride does not affect this conformation.

Unfortunately, the target molecules could not be prepared because the chiral auxiliaries (8-phenyl- and 8-(β-naphtyl)menthol) could not be removed from the hydroxylamine derivatives **10** and **11**.

Various transesterfication methods such as $MeOH/H_2SO_4$, reflux; $Ti(OiC_3H_7)_4/EtOH$, reflux; MeOH/HCl gave starting material or unidentified products.

Moreover, it was also impossible to establish the absolute configuration of both diastereoisomers of **10** and **11** by X-ray analysis as all crystallization attempts failed. However, according to the models as depicted in Chart I attack on the *re* face of the oxime unit has to be expected, resulting in an S-configuration at the α-carbon atom of the major isomer.

In conclusion, the asymmetric reduction of the oxime double bond is feasible. However, it has not yet yielded an approach to optically pure N-hydroxytryptophan as removal of the chiral auxiliary has not been achieved so far. The excellent asymmetric induction recently observed by Corey in the reduction of ketones with borane in the presence of a catalytic amount of chiral oxazaborolidines[15] makes a study of the approach via chiral reducing agents (vide supra) more worthwhile.

Experimental Section

Melting points were taken on a Koefler hot stage (Leitz-Wetzlar) and are uncorrected. Ultraviolet spectra were measured with a Perkin Elmer spectrometer, Model lambda 5. Proton magnetic resonance spectra were measured on a Bruker WH-90 or on a Bruker AM 400 spectrometer. Chemical shifts are reported as δ-values relative to tetramethylsilane as an internal standard; deuteriochloroform was used as solvent unless stated otherwise. Mass spectra were obtained with a double-focusing VG 7070E spectrometer. Thin-layer chromatography (TLC) was carried out by using silica gel F-254 plates (thickness 0.25 mm). Spots were visualized with a UV hand lamp, iodine vapor, or Cl_2-TDM.[16] For column chromatography Merck silica gel (type 60H) was used.

α-(Hydroxyimino)-β-indol-3-ylpropanoate (12)

A solution of 2N NaOH (8 mL) was added to a stirred solution of **2**[1] (2.48 g, 10 mmol)in dioxane (20 mL). Stirring was continued aEBt room temperature under argon for 3 days after which the reaction mixture was neutralized with 4N HCl and washed with ethyl acetate. The organic layer was washed with brine, dried ($MgSO_4$) and concentrated to dryness. The residue was crystallized from $MeOH/H_2O$ to give 1.05 g (70%) of **12**. mp 151-153°C; Rf 0.00 ($CHCl_3$/MeOH, 93/7, v/v); EIMS (70 eV) m/z (relative intensity) 218 (M^+, 20), 201 ($[M\text{-}OH]^+$, 8), 156 ($[C_{10}H_8N_2]^+$, 80), 155 ($[C_{10}H_7N_2]^+$, 100); ^1H NMR ($CDCl_3/CD_3OD$) δ 8.93 (br s, 1H, NH), 7.80-6.97 (m, 4H, indole C(4)-C(7)H), 7.00 (s, 1H, indole C(2)H)m 4.06 (s, 2H, indole C(3)CH_2); Anal. Calcd. for $C_{11}H_{10}N_2O_3$ (Mw 218.215) :C, 60.55; H, 4.62; N, 12.84. Found: C, 59.97; H, 4.56; N, 12.64.

Ethyl α-(3-propeneoximino)-β-indol-3-ylpropanoate (14)

To a stirred solution of **2**[1] (5 g, 20 mmol) and allylbromide (5g, 40 mmol) in DMSO 50 mL) was added portionwise KO*t*Bu (2.46 g, 22 mmol) at room temperature. After completion of the reaction (2d.) as was monitored by TLC ($CHCl_3$/MeOH, 99/1, v/v) dichloromethane was added (150 mL). The reaction mixture was washed with water (3x 200 mL) and 1N HCl. The organic layer was washed with brine, dried (Na_2SO_4) and concentrated to dryness. The residue was subjected to column chromatography ($CHCl_3$) to give 4.9 g (85%) of **14**. Oil; Rf 0.64 ($CHCl_3$/MeOH, 99/1, v/v); EIMS (70 eV) m/z (relative intensity) 286 (M^+, 26), 229 ($[M\text{-}C_3H_5O]^+$, 32), 155 (71), 130 ($[C_9H_8N]^+$, 100); ^1H NMR δ 8.06 (br s, 1H, NH), 7.77-6.98 (m, 5H, indole C(4)-C(7)H and C(2)H), 6.29-5.84 (m, 1H, OCH_2CH=), 5.43-5.18 (m, 2H, CH=CH_2), 4.81 (dd, 2H, $NOCH_2$), 4.27 (q, 2H, OCH_2CH_3), 4.09 (s, 2H, indole C(3)CH_2), 1.26 (t, 3H, OCH_2CH_3).

α-(3-propeneoximino)-β-indol-3-ylpropanoate (15)

The same procedure was followed as described for **12**. Reaction of **14** (2.33 g, 8.1 mmol) gave after column chromatography ($CHCl_3$/MeOH/HOAc, 87/10/3, v/v/v) 2.1 g (96%) of **15**. Oil; Rf 0.47 ($CHCl_3$/MeOH/HOAc, 87/10/3, v/v/v); EIMS (70 eV) m/z (relative intensity) 258 (M^+, 29), 201 ($[M\text{-}C_3H_5O]^+$, 15), 155 (92), 130 ($[C_9H_8N]^+$, 100); ^1H NMR δ 8.04 (br s, 1H, NH), 7.81-7.04 (m, 5H, indole C(4)-C(7)H and C(2)H), 6.27-5.82 (m, 1H, OCH_2CH=), 5.47-5.26 (m, 2H, CH=CH_2), 4.82 (dd, 2H, $NOCH_2$), 4.33 (br s, 1H, OH), 4.08 (s, 2H, indole C(3)CH_2).

(-)-Menthyl α-(3-propeneoximino)-β-indol-3-ylpropanoate (16)
To a stirred solution of **15** (45 mg, 0.17 mmol), (-)-menthol (27 mg, 0.17 mmol) and DMAP (0.1 equiv.) in acetonitrile (5 mL), DCC (36 mg, 0.17 mmol) was added at room temperature under argon atmosphere. After completion of the reaction (24h) as was monitored by TLC (EtOAc/n-hexane, 35/65, v/v) the reaction mixture was filtered and the filtrate concentrated to dryness. The residue was subjected to column chromatography (EtOAc/n-hexane, 10/90, v/v) to give 61 mg (90%) of **16**. Oil; Rf 0.62 (EtOAc/n-hexane, 35/65, v/v); CIMS (100 eV) m/z (relative intensity) 357 ($[M+1]^+$, 14), 396 (M^+, 12), 341 (16), 201 (79), 130 ($[C_9H_8N]^+$, 100); ^{1}H NMR δ 7.97 (br s, 1H, NH), 7.78-7.03 (m, 4H, indole C(4)-C(7)H), 7.10 (s, 1H, indole C(2)H), 6.27-5.95 (m, 1H, OCH_2CH=), 5.40-5.18 (m, 2H, CH=CH_2), 4.98-4.73 (m, 1H, menthol C(1)H), 4.10 (s, 2H, indole C(3)CH_2), 2.10-1.00 (m, 9H, menthol C(2)H, C(3)H_2- C(4)H_2, C(5)H, C(6)H_2 and C(8)H), 0.90 (d, 3H, J=6.1Hz, C(8)CH_3), 0.81 (d, 3H, J=6.8Hz, C(8)CH_3), 0.68 (d, 3H, J=6.7Hz, C(5)CH_3)

(-)-8-Phenylmenthyl α-(3-propeneoximino)-β-indol-3-ylpropanoate (17)
The same procedure was followed as described for **16**. Reaction of **15** (500 mg, 1.94 mmol), (-)-8-phenylmenthol (Aldrich: 494 mg, 2.13 mmol), DCC (439 mg, 2.13 mmol) and DMAP (24 mg, 0.2 mmol) gave after column chromatography (n-hexane/EtOAc, 90/10, v/v) 708 mg (77%) of **17**. Oil; Rf 0.48 (n-hexane/EtOAc, 2/1, v/v); $[\alpha]^{22}_D$ +105[2] (c=1.55, methanol); EIMS (70 eV) m/z (relative intensity) 472 (M^+, 31), 201 (71), 155 (52), 130 ($[C_9H_8N]^+$, 100); ^{1}H NMR δ 7.97 (br s, 1H, NH), 7.70-6.96 (m, 10H, indole C(4)-C(7)H, C(2)H and C_6H_5), 6.28-5.85 (m, 1H, OCH_2CH=), 5.43-5.21 (m, 2H, CH=CH_2), 4.87 (dt, 1H, menthol C(1)H), 4.78 (dd, 2H, $NOCH_2$), 3.92 and 3.46 (AB spectrum, 2H, ^{2}J=14.0Hz, indole C(3)CH_2), 2.11 (br t, 1H, menthol C(2)H), 1.78-0.60 (m, 7H, menthol C(3)-C(4)H_2, C(5)H and C(6)H_2), 1.23 (s, 6H, C(8)$(CH_3)_2$), 0.69 (d, 3H, C(5)CH_3).

(-)-8-(β-Naphtyl)menthyl α-(3-propeneoximino)-β-indol-3-ylpropanoate (19)
The same procedure was followed as described for **16**. Reaction of **15** (500 mg, 1.94 mmol), (-)-8-(β-naphtyl)menthol[8] (551 mg, 1.95 mmol), DCC (439 mg, 2.13 mmol) and DMAP (24 mg, 0.2 mmol) gave after column chromatography (n-hexane/EtOAc, 90/10, v/v) 795 mg (78%) of **18**. Oil; Rf 0.33 (EtOAc/n-hexane, 1/4, v/v); EIMS (70 eV) m/z (relative intensity) 522 (M^+, 20), 201 (24), 169 ($[C_{13}H_{13}]^+$, 100), 155 ($[C_{12}H_{11}]^+$, 60), 141 ($[C_{11}H_9]^+$, 34), 130 ($[C_9H_8N]^+$, 44); ^{1}H NMR 7.91 (br s, 1H, NH), 7.80-6.87 (m, 12H, indole C(4)-C(7)H, C(2)H and β-naphtyl), 6.18-5.76 (m, 1H, OCH_2CH=), 5.36-5.16 (m, 2H, CH=CH_2), 4.98 (dt, 1H, menthol C(1)H), 4.59 (dd, 2H, $NOCH_2$), 3.54 and 3.13 (AB spectrum, 2H, ^{2}J=14.9Hz, indole C(3)CH_2), 2.17 (dt, 1H, menthol C(2)H), 1.78-0.69 (m, 7H, menthol C(3)-C(4)H_2, C(5)H and C(6)H_2), 1.36 and 1.29 (2xs, 6H, C(8)$(CH_3)_2$), 0.80 (d, 3H, C(5)CH_3).

(-)-Menthyl α-(hydroxyimino)-β-indol-3-ylpropanoate (6)
Procedure A: Titanium(IV)isopropoxide (734 mg, 2.6 mmol) was added to a stirred solution of **2**[1] (4.92 g, 20 mmol) and (-)-menthol (15.6 g, 100 mmol) in dioxane (1.5 mL) at 85°C under an argon atmosphere. After completion of the reaction (24 h) as was monitored by TLC (n-hexane/EtOAc, 70/30, v/v) the solvent was evaporated in vacuo. The residue was dissolved in dichloromethane and washed successively with 1n HCl, 0.1 N $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and concentrated to dryness. The residue was subjected to column chromatography (n-hexane/EtOAc, 20/80, v/v) to give 6.09 g (85%) of **6**. Crystallized from CH_2Cl_2/n-hexane: mp 151-152°C; Rf 0.52 (n-hexane/EtOAc, 70/30, v/v); $[\alpha]^{22}_D$ -59° (c=2.3, methanol); CIMS (100 eV) m/z (relative intensity) 357 ($[M+1]^+$, 14), 356 (M^+, 22), 341 (16), 219 (79), 130 ($[C_9H_8N]^+$, 100); ^{1}H NMR δ 7.99 (br s, 1H, NH), 7.80-7.02 (m, 4H, indole C(4)-C(7)H), 7.10 (s, 1H, indole C(2)H), 4.98-4.70 (m, 1H, menthol C(1)H), 4.09 (s, 2H, indole C(3)CH_2), 2.10-1.00 (m, 9H, menthol C(2)H, C(3)H_2- C(4)H_2, C(5)H, C(6)H_2 and C(8)H), 0.91 (d, 3H, J=6.1Hz, C(8)CH_3), 0.79 (d, 3H, J=6.8Hz, C(8)CH_3), 0.65 (d, 3H, J=6.7Hz, C(5)CH_3); Anal. Calcd. for $C_{21}H_{28}N_2O_3$ (Mw 356.468): C, 69.74; H, 7.92; N, 7.86. Found: C, 69.81; H, 7.84; N, 7.73.
Procedure B: A solution of **16** (50 mg, 0.125 mmol), $Pd(OAc)_2$ (0.28 mg, 0.00125 mmol), PPh_3 (1.3 mg, 0.005 mmol) and $HCOONHEt_3$ (160 mg, 0.375 mmol) in 80% aqueous acetonitrile was refluxed. After completion of the reaction (1h) as was monitored by TLC ($CHCl_3$/MeOH, 97/3, v/v) the reaction mixture was diluted with ethyl acetate and subsequently washed with brine. The organic layer was dried ($MgSO_4$) and the solvent evaporated in vacuo. The residue was subjected to column chromatography ($CHCl_3$/MeOH, 97/3, v/v) to give 40 mg (89%) of **6**.

(-)-8-Phenylmenthyl α-(hydroxyimino)-β-indol-3-ylpropanoate (7)

Procedure B was followed as described for **6**. Reaction of **17** (500 mg, 1.06 mmol), $Pd(OAc)_2$ (2.37 mg, 0.0106 mmol), PPh_3 (11 mg, 0.0424 mmol) and $HCOONHEt_3$ (1.3 mg, 3.18 mmol) gave after column chromatography (EtOAc/n-hexane, 20/80, v/v) 440 mg (96%) of **7**. White solid material. Crystallization attempts failed. Rf 0.26 (EtOAc/n-hexane, 1/2, v/v), $[\alpha]^{22}_D$ +86[3] (c=1.75, methanol); EIMS (70 eV) m/z (relative intensity) 432 (M^+, 45), 218 (39), 201 (94), 155 (37), 130 ($[C_9H_8N]^+$, 100); ^{1}H NMR δ 7.98 (br s, 1H, NH), 7.81-7.00 (m, 10H, indole C(4)-C(7)H, C(2)H and C_6H_5), 4.98 (dt, 1H, menthol C(1)H), 3.97 and 3.57 (AB spectrum, 2H, ^{2}J=14.1Hz, indole C(3)CH_2), 2.05 (br t, 1H, menthol C(2)H), 1.91-0.70 (m, 7H, menthol C(3)-C(4)H_2, C(5)H and C(6)H_2), 1.24 and 1.19 (2xs, 6H, C(8)$(CH_3)_2$), 0.80 (d, 3H, C(5)CH_3).

(-)-8-(β-Naphtyl)menthyl α-(hydroxyimino)-β-indol-3-ylpropanoate (8)

Procedure B was followed as described for **6**. Reaction of **18** (740 mg, 1.41 mmol), $Pd(OAc)_2$ (6.3 mg, 0.028 mmol), PPh_3 (30 mg, 0.11 mmol) and $HCOONHEt_3$ (1.8 mg, 4.16 mmol) gave after column chromatography (EtOAc/n-hexane, 20/80, v/v) 680 mg (100%) of **8**. White solid material. Crystallization attempts failed. Rf 0.81 (EtOAc/n-hexane, 1/1, v/v); EIMS (70 eV) m/z (relative intensity) 482 (M^+, 16), 202 (28), 201 (27), 169 ($[C_{13}H_{13}]^+$, 100), 155 ($[C_{12}H_{11}]^+$, 48), 141 ($[C_{11}H_9]^+$, 24), 130 ($[C_9H_8N]^+$, 50); ^{1}H NMR 7.92 (br s, 1H, NH), 7.79-6.96 (m, 12H, indole C(4)-C(7)H, C(2)H and β-naphtyl), 5.04 (dt, 1H, menthol C(1)H), 3.60 and 3.22 (AB spectrum, 2H, ^{2}J=14.0Hz, indole C(3)CH_2), 2.16 (dt, 1H, menthol C(2)H), 1.78-0.69 (m, 7H, menthol C(3)-C(4)H_2, C(5)H and C(6)H_2), 1.36 and 1.29 (2xs, 6H, C(8)$(CH_3)_2$), 0.84 (d, 3H, C(5)CH_3).

(-)-Menthyl α-(hydroxyamino)-β-indol-3-ylpropanoate (9)

A solution of HCl in dioxane (37 mL of a 7N solution) was added dropwise to a stirred solution of **6** (5.43 g, 15.3 mmol) and $Me_3N.BH_3$ (1.22 g, 16.8 mmol) in dioxane (60 mL) at room temperature under an argon atmosphere. Stirring was continued for 2h. The reaction mixture was concentrated to dryness; the residue dissolved in dichloromethane; the solution was neutralized with 0.1 N $NaHCO_3$, washed with brine and dried with Na_2SO_4. The residue obtained after evaporation of the solvent in vacuo, was subjected to column chromatography ($CHCl_2$/MeOH, 98.5/1.5, v/v) to give 4.66 g (85%) of **9**. The two diastereomers in a ratio of 1/1[12] could not be separated. White solid. Rf 0.43 ($CHCl_3$/MeOH, 93/7, v/v); CIMS (100 eV) m/z (relative intensity) 359 ($[M+1]^+$, 39), 343 ($[M-OH]^+$, 60), 221 (54), 130 ($[C_9H_8N]^+$, 100); ^{1}H NMR of the mixture of diastereomers δ 8.05 (br s, 1H, NH), 7.67-7.05 (m, 5H, indole C(2)H and indole C(4)-C(7)H), 5.29 (br s, 1H, HNOH of diastreomer a), 5.19 (br s, 1H, HNOH of diastereomer b), 4.91-4.59 (m, 1H, menthol C(1)H), 3.97 (X part of ABX spectrum, 1H, indole C(3)CH_2**CH**), 3.18-3.05 (AB part of ABX spectrum, 2H, ^{2}J=14.4Hz, J=4.4Hz, J=8.9Hz, indole C(3)CH_2), 2.10-1.00 (m, 9H, menthol C(2)H, C(3)-C(4)H_2, C(5)H ,C(6)H_2 and C(8)H), 0.89-0.62 (m, 9H, menthol C(5)CH_3 and C(8)$(CH_3)_2$).

(-)-8-Phenylmenthyl α-(hydroxyamino)-β-indol-3-ylpropanoate (10)

The same procedure was followed as described for **9**. Reaction of **7** (385 mg, 0.89 mmol) and $Me_3N.BH_3$ (65 mg, 0.9 mmol) gave after column chromatography (EtOAc/n-hexane, 1/2, v/v) 71 mg (18%) of diastereomer **a** and 206 mg (54%) of diastereomer **b**.

Diastereomer a: White solid. Crystallization attemps failed. Rf 0.63 (EtOAc/n-hexane, 1/1, v/v); EIMS (70 eV) m/z (relative intensity) 434 (M^+, 4), 416 ($[M-H_2O]^+$, 10), 202 (40), 130 ($[C_9H_8N]^+$, 100), 119 ($[C_9H_{11}]^+$, 94); ^{1}H NMR δ 8.67 (br s, 1H, NH), 7.54-6.89 (m, 10H, indole C(2)H and C(4)-C(7)H and C_6H_5), 6.71 (br s, 2H, HNOH), 4.91 (dt, 1H, menthol C(1)H), 3.55 (br t, 1H, indole C(3)CH_2**CH**), 3.04 (br d, 2H, indole C(3)CH_2), 2.13-1.00 (m, 8H, menthol C(2)H, C(3)-C(4)H_2, C(5)H and C(6)H_2), 1.24 and 1.13 (2xs, 6H, C(8)$(CH_3)_2$), 0.86 (br d, 3H, C(5)CH_3).

Diastereomer b: White solid. Crystallization attemps failed. Rf 0.52 0.63 (EtOAc/n-hexane, 1/1, v/v); $[\alpha]^{22}_D$ +5[5] (c=2.2, methanol); EIMS (70 eV) m/z (relative intensity) 434 (M^+, 2), 416 ($[M-H_2O]^+$, 6), 202 (26), 130 ($[C_9H_8N]^+$, 100), 119 ($[C_9H_{11}]^+$, 59); ^{1}H NMR δ ^{1}H NMR δ 8.43 (br s, 1H, NH), 7.49-6.99 (m, 10H, indole C(2)H and C(4)-C(7)H and C_6H_5), 6.53 (br s, 2H, HNOH), 4.80 (dt, 1H, menthol C(1)H), 3.46 (br t, 1H, indole C(3)CH_2**CH**), 2.93 (br d, 2H, indole C(3)CH_2), 2.11-0.61 (m, 8H, menthol C(2)H, C(3)-C(4)H_2, C(5)H and C(6)H_2), 1.31 and 1.21 (2xs, 6H, C(8)$(CH_3)_2$), 0.83 (br d, 3H, C(5)CH_3).

(-)-8-(β-Naphtyl)menthyl α-(hydroxyamino)-β-indol-3-ylpropanoate (11)

The same procedure was followed as described for **9**. Reaction of **8** (700 mg, 1.45 mmol) and $Me_3N.BH_3$ (210 mg, 2.92 mmol) gave after column chromatography ($CHCl_3$/MeOH, 99/1, v/v) 86 mg (12%) of diastereomer **a** and 463 mg (66%) of diastereomer **b**.

Diastereomer a: White solid. Crystallization attemps failed. Rf 0.21 (EtOAc/n-hexane, 1/2, v/v); $[\alpha]^{22}_D$ -27[4] (c=1.9, methanol); EIMS (70 eV) m/z (relative intensity) 466 (M^+, 5), 277 (15), 202 (43), 169 ($[C_{13}H_{13}]^+$, 71); 155 ($[C_{12}H_{11}]^+$, 37), 141 ($[C_{11}H_9]^+$, 18), 130 ($[C_9H_8N]^+$, 100); ^{1}H NMR δ 7.96 (br s, 1H, NH), 7.80-7.01 (m, 11H, indole C(2)H and C(4)-C(7)H and β-naphtyl), 6.72 (d, 1H, naphtyl αH), 5.00 (dt, 1H, menthol C(1)H), 3.23 (X part of a ABX spectrum, 1H, indole $C(3)CH_2CH$), 2.87 and 2.77 (AB part of ABX spectrum, 2H, ^{2}J=14.4Hz, J=4.3 Hz, J=10.0 Hz, indole $C(3)CH_2$), 2.21 (dt, 1H, menthol C(2)H), 2.04-0.61 (m, 7H, menthol C(3)-C(4)H_2, C(5)H and C(6)H_2), 1.38 and 1.26 (2xs, 6H, $C(8)(CH_3)_2$), 0.88 (d, 3H, $C(5)CH_3$).

Diastereomer b: White solid. Crystallization attemps failed. Rf 0.16 (EtOAc/n-hexane, 1/2, v/v); $[\alpha]^{22}_D$ -3[6] (c=1.95, methanol); EIMS (70 eV) m/z (relative intensity) 466 (M^+, 17), 202 (53), 169 ($[C_{13}H_{13}]^+$, 69); 155 ($[C_{12}H_{11}]^+$, 36), 141 ($[C_{11}H_9]^+$, 20), 130 ($[C_9H_8N]^+$, 100); ^{1}H NMR δ 7.93 (br s, 1H, NH), 7.82-7.02 (m, 11H, indole C(2)H and C(4)-C(7)H and β-naphtyl), 6.65 (d, 1H, naphtyl αH), 4.91 (dt, 1H, menthol C(1)H), 3.08 (X part of a ABX spectrum, 1H, indole $C(3)CH_2CH$), 2.77 and 2.70 (AB part of ABX spectrum, 2H, ^{2}J=14.7Hz, J=5.7 Hz, J=8.3 Hz, indole $C(3)CH_2$), 2.18 (dt, 1H, menthol C(2)H), 1.91-0.60 (m, 7H, menthol C(3)-C(4)H_2, C(5)H and C(6)H_2), 1.44 and 1.29 (2xs, 6H, $C(8)(CH_3)_2$), 0.84 (d, 3H, $C(5)CH_3$).

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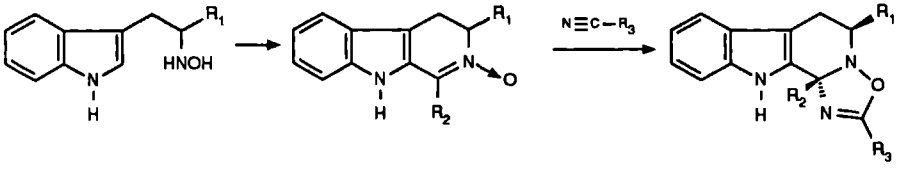
CHAPTER 3

Syntheses of 3,4-dihydro-β-carboline nitrones from N-hydroxy-tryptophan and -tryptamine derivatives and their 1,3-dipolar cycloaddition with nitriles.



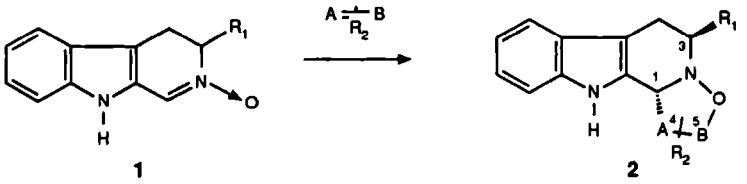
"1,3-Dipolar Cycloaddition of Nitrones with Nitriles. Scope and Mechanistic Study" Pedro H.H. Hermkens, Jan H. v. Maarseveen, Chris G. Kruse, Hans W. Scheeren, *Tetrahedron* (**1989**), *30*, 5009.

Chapter 3.1

INTRODUCTION

Compounds containing the β-carboline structure have aroused considerable interest in neuropharmacology. For two reasons we wanted to investigate the N(2)-oxo-3,4-dihydro-β-carbolines (Scheme I, *e.g.* **1**). The first is the *in vitro* affinity of these compounds towards the benzodiazepine receptor[1]. The second reason is that the nitrone functionality of these compounds can undergo [3+2] cycloaddition reactions with a variety of dipolarophiles[2]. The result would be a 5-membered ring containing two (N-O) or more hetero atoms annulated with the β-carboline fragment (Scheme I, *e.g.* **2**). Compounds having the general structure **2** might well have a biological activity due to their structural similarity with several classes of active indole alkaloids.

Scheme I



1 **2**

Recently, we demonstrated that the 1,3-dipolar cyloaddition of the nitrone **1** (R_1=COOEt) with alkenes[3] and nitriles[4] proceeds with high or complete regio- and stereoselectivity resulting in isoxazolidines and Δ^4-1,2,4-oxadiazolines, respectively. In chapter 4 the application of this cycloaddition to yield an isoxazolidine in the total synthesis of natural products of the fumitremorgin-verruculogen group is reported.

In chapter 3.2 the syntheses of 3,4-dihydro-β-carboline nitrones is described and in chapter 3.3 the cycloaddition of some of these nitrones with nitriles is discussed in more detail.

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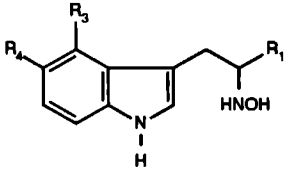
2. For reviews see: a) Black, D.St.C.; Crozier, F.F.; Davies, V.C. *Synthesis* **1975**, 205. b) Breuer, E. in *The Chemistry of Functional Groups Supp. F, The Chemistry of Amino and Nitro Compounds and their Derivatives*, S. Patai, Ed.; J.Wiley, Chichester, **1982**, Chapter 13, p. 45. c) Tufariello, J.J. in *1,3-Dipolar Cycloaddition Chemistry*, Ed. A. Padwa, J.Wiley, Chichester, **1984**, Vol. 2, Chapter 9, p. 83.
3. Plate, R.; Hermkens, P.H.H.; Smits, J.M.M.; Ottenheijm, H.C.J. *J.Org.Chem.* **1986**, *51*, 309.
4. Plate, R.; Hermkens, P.H.H.; Smits, J.M.M.; Nivard, R.J.F.; Ottenheijm, H.C.J. *J.Org.Chem.* **1987**, *52*, 1047.

Chapter 3.2

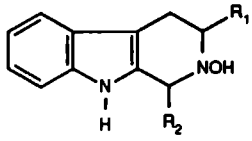
SYNTHESES OF N-OXO-3,4-DIHYDRO-β-CARBOLINES FROM N-HYDROXY-TRYPTOPHAN AND -TRYPTAMINE DERIVATIVES.

As part of synthetic studies on N-hydroxytryptophan derivatives it was found that **1** can be converted into the N-hydroxy-tetrahydro-β-carboline **6** (Scheme I).[1,2] In addition, it was described[1] that the nitrone **8** could be prepared either by DDQ oxidation of **6** or -more efficiently- in a single step from **1** by acid-catalyzed condensation with trimethyl orthoformate.

Scheme I

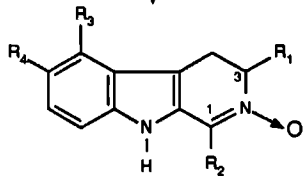


	R_1	R_3	R_4
1	COOEt	H	H
2	COOEt	Cl	H
3	COOEt	H	Cl
4	CH_3	H	H
5	H	H	H



6: R_1=COOEt, R_2=H
7: R_1=CH_3, R_2=Ph

	R_1	R_2	R_3	R_4
8	COOEt	H	H	H
9	COOEt	H	Cl	H
10	COOEt	H	H	Cl
11	CH_3	H	H	H
12	H	H	H	H
13	CH_3	Ph	H	H

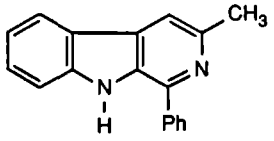


i) R_2CHO / TFA, CH_2Cl_2 ii) DDQ / CH_2Cl_2 iii) $HC(OMe)_3$ / TFA

Following the latter procedure the N-hydroxy compounds **2-5** (chapter 2.1) were converted into the C(1) unsubstituted nitrones **9-12** in 75%, 67%, 90% and 31%[3], respectively.

The C(1) substituted derivative **13** was prepared from 1-phenyl-2-hydroxy-3-methyl-1,2,3,4-tetrahydro-β-carboline (**7**) -derived from **4** and benzaldehyde in the presence of TFA[4]- by DDQ oxidation. A draw-back of this approach is that not only **13**, but also **14**(18%) and **15**(6%) were formed as a result of overoxidation (partly accompanied by dehydrogenation (Chart I)).

Chart I



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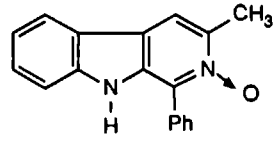

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Experimental Section

Melting points were taken on a Koefler hot stage (Leitz-Wetzlar) and are uncorrected. Ultraviolet spectra were measured with a Perkin Elmer spectrometer, Model lambda 5. Proton magnetic resonance spectra were measured on a Bruker WH-90 or on a Bruker AM 400 spectrometer. Chemical shifts are reported as δ-values relative to tetramethylsilane as an internal standard; deuteriochloroform was used as solvent unless stated otherwise. Mass spectra were obtained with a double-focusing VG 7070E spectrometer. Thin-layer chromatography (TLC) was carried out by using silica gel F-254 plates (thickness 0.25 mm). Spots were visualized with a UV hand lamp, iodine vapor, or Cl_2-TDM.[5] For column chromatography Merck silica gel (type 60H) was used.

2-Oxo-3-(ethoxycarbonyl)-5-chloro-3,4-dihydro-β-carboline (9). Trifluoracetic acid (0.45 mL) was added dropwise to a stirred solution of **2** (1.0 g., 3.6 mmol) in $HC(OMe)_3$ (10 mL) at room temperature and in argon atmosphere. Stirring was continued for 2.5 h. The solution was then concentrated to near dryness, dissolved in CH_2Cl_2 and concentrated again. The residue was dissolved in CH_2Cl_2 and washed with $NaHCO_3$ and water and dried over Na_2SO_4. Evaporation of the solvent gave crystalline **9**, which was recrystallized (CH_2Cl_2/MeOH/n-hexane) to yield 0.78 g. (75%) **9**: mp 173°C (decomposes); Rf 0.28 (solvent system D); UV (MeOH) λ_{max} 218, 362, 367 nm, λ_{min}; EIMS (70 eV) m/z 294 ($[M+2]^+$, 16%)· 292 (M^+, 27%), 276 ($[C_{14}H_{11}ClN_2O_2]^+$, 8%), 274 ($[C_{14}H_{11}ClN_2O_2]^+$,14%), 221 ($[C_{11}H_8ClN_2O]^+$, 41%), 219($[C_{11}H_8ClN_2O]^+$, 72%), 204 ($[C_{11}H_7N_2Cl]^+$, 46%), 202 ($[C_{11}H_7N_2Cl]^+$, 100%); ^{1}H NMR ($CDCl_3/CD_3OD$,95/5,v/v) δ 8.01 (s, 1H, C(1)H), 7.32-7.00 (m, 3H, indole C(6)-C(8)H), 4.88 (X part of ABX spectrum, 1H,^{3}J 3.2 Hz, ^{3}J 8.2 Hz, C(3)H), 4.22 (q, 2H,^{3}J=7.1 Hz, OCH_2CH_3), 4.11-3.72 (AB part of ABX spectrum, 2H,^{2}J 17.7 Hz, ^{3}J 3.2 Hz, ^{3}J 8.2 Hz, C(4)H), 1.21 (t, 3H, ^{3}J 7.1 Hz, OCH_2CH_3).

2-oxo-3-(ethoxycarbonyl)-6-chloro-3,4-dihydro-β-carboline (10). The same procedure was followed as described for **9**. Reaction of **3** (1.0 g, 3.6 mmol) in $HC(OMe)_3$ (10 mL) and TFA (0.5 mL) gave after column chromatography ($CHCl_3$/MeOH, 93/7, v/v) in 67% yield **10**. Recrystallized from CH_2Cl_2/MeOH/n-hexane: mp 208°C (decomposes); Rf 0.19 (solvent system D); UV (MeOH) λ_{max} 218, 362, 367 nm, λ_{min}; EIMS (70 eV) m/z 294 ($[M+2]^+$, 16%)· 292 (M^+, 27%), 276 ($[C_{14}H_{11}ClN_2O_2]^+$, 8%), 274 ($[C_{14}H_{11}ClN_2O_2]^+$,14%), 221 ($[C_{11}H_8ClN_2O]^+$, 41%), 219($[C_{11}H_8ClN_2O]^+$, 72%), 204 ($[C_{11}H_7N_2Cl]^+$,46%), 202 ($[C_{11}H_7N_2Cl]^+$, 100%); ^{1}H NMR ($CDCl_3/CD_3OD$,95/5,v/v) δ 7.96 (s, 1H, C(1)H), 7.44-7.09 (m, 3H, indole C(5)H and C(7)-C(8)H), 4.94-4.82 (X part of ABX spectrum, 1H, C(3)H), 4.21 (q, 2H,^{3}J 7.1 Hz, OCH_2CH_3), 3.59-3.53 (AB part of ABX spectrum, 2H, C(4)H), 1.24 (t, 3H, ^{3}J 7.1 Hz, OCH_2CH_3).

2-oxo-3-methyl-3,4-dihydro-β-carboline (11)

The same procedure was followed as described for **9**. Reaction of **4** (2 g, 10.5 mmol) in $HC(OMe)_3$ (25 mL) and TFA (1.5 mL) gave after column chromatography ($CHCl_3$/MeOH, 93/7, v/v) 1.9 g (90%) of **11**. Recrystallized from CH_2Cl_2/n-hexane: mp 214-216°C; Rf 0.21 ($CHCl_3$/MeOH, 93/7, v/v); UV (methanol) λmax 212, 262, 351 nm; EIMS (70 eV) m/z (relative intensity) 200 (M^+, 100), 183 ($[M-OH]^+$, 37), 169 ($[C_{11}H_9N_2]^+$, 29); ^{1}H NMR δ 9.58 (br s, 1H, NH), 7.81 (s, 1H, C(1)H), 7.50-7.03 (m, 4H, C(5)-C(8)H), 4.30 (m, 1H, C(3)H)), 3.38 and 2.94 (AB part of ABX spectrum, 2H, ^{2}J-17.0Hz, J=6.8Hz, J=4.5Hz, C(4)H_2), 1.56 (d, 3H, C(3)HCH_3).

2-oxo-3,4-dihydro-β-carboline (12)

The same procedure was followed as described for **9**. Reaction of **3** (1 g, 5.67 mmol) in $HC(OMe)_3$ (25 mL) and TFA (6.5 g, 56.7 mmol) gave after column chromatography ($CHCl_3$/MeOH, 93/7, v/v) 330 mg (31%) of **9**. Crystallization attempts failed. Rf 0.24 ($CHCl_3$/MeOH, 90/10, v/v); CIMS (100 eV) m/z (relative intensity) 187 ($[M+1]^+$, 33), 170 ($[C_{11}H_{10}N_2]^+$, 100), 169 ($[C_{11}H_9N_2]^+$, 75); ^1H NMR δ 9.00 (br s, 1H, NH), 7.80 (s, 1H, C(1)H), 7.54-7.09 (m, 4H, C(5)-C(8)H), 4.26 (t, 2H, J=1.9 Hz, $C(3)H_2$), 3.21 (t, 2H, J=1.9Hz, $C(4)H_2$).

1-phenyl-2-oxo-3-methyl-3,4-dihydro-β-carboline (13); 1-phenyl-3-methyl-β-carboline (14); 1-phenyl-2-oxo-3-methyl-β-carboline (15)

To a stirred solution of **7**[4] (2.58 g, 9.3 mmol) in dichloromethane (100 mL) was added at room temperature dropwise a solution of DDQ (2.1 g, 9.3 mmol) in dichloromethane (100 mL). After completion of the reaction (1h.) as was monitored by TLC ($CHCl_3$/MeOH, 93/7, v/v) the reaction mixture was washed with 0.1 N NaOH and brine. The organic layer was dried (Na_2SO_4) and the solvent evaporated in vacuo. The residue was subjected to column chromatography ($CHCl_3$/MeOH, 99/1, v/v) to give 430 mg (18%) of **14**, 1.62 g (63%) of **13** and 160 mg (6%) of **15**.

Compound 13: Recrystallized from CH_2Cl_2/n-hexane: mp 243-245°C; Rf 0.21 ($CHCl_3$/MeOH, 93/7, v/v); UV (methanol) λmax 212, 255, 281, 352 nm; EIMS (70 eV) m/z (relative intensity) 276 (M^+, 100), 259 ($[M\text{-}OH]^+$, 31), 245 ($[C_{17}H_{13}N_2]^+$, 20); ^1H NMR δ 7.83-7.09 (m, 10H, NH, C(5)-C(8)H and C_6H_5), 4.44 (m, 1H, C(3)H), 3.53 and 2.97 (AB part of ABX spectrum, 2H, ^2J=16.5Hz, J=7.0Hz, J=4.3Hz, $C(4)H_2$), 1.61 (d, 3H, $C(3)HCH_3$); Anal. Calcd. for $C_{18}H_{16}N_2O$ (Mw 276.341): C, 78.24; H, 5.84; N, 10.14. Found: C, 78.21; H, 5.79; N, 10.04.

Compound 14: Recrystallized from CH_2Cl_2/n-hexane: mp 178-179°C; Rf 0.48 ($CHCl_3$/MeOH, 93/7, v/v); UV (methanol) λmax 220, 236, 285, 295, 356, 365 nm; EIMS (70 eV) m/z (relative intensity) 258 (M^+, 100); ^1H NMR δ 8.33 (br s, 1H, NH), 8.14-7.16 (m, 10H, C_6H_5, C(5)-C(8)H and C(4)H), 1.64 (s, 3H, $C(3)CH_3$); Anal. Calcd. for $C_{18}H_{14}N_2$ (Mw 258.326): C, 83.69; H, 5.46; N, 10.84. Found: C, 83.21; H, 5.44; N, 10.76.

Compound 15: Recrystallized from CH_2Cl_2/n-hexane: mp 210-212°C; Rf 0.11 ($CHCl_3$/MeOH, 93/7, v/v); UV (methanol) λmax 234, 260, 322, 348 nm; EIMS (70 eV) m/z (relative intensity) 274 (M^+, 62), 257 ($[M\text{-}OH]^+$, 100); ^1H NMR δ 8.20 (br s, 1H, NH), 8.00-7.12 (m, 10H, C_6H_5, C(5)-C(8)H and C(4)H), 1.69 (s, 3H, $C(3)CH_3$)

References and Notes

1. Plate, R.; Hermkens, P.H.H.; Smits, J.M.M.; Ottenheijm, H.C.J. *J.Org.Chem.* **1986**, *51*, 309.
2. See also chapter 5.
3. The yield of nitrone **12** was low because under the reaction conditions **12** as well as the N-hydroxy compound **5** were unstable.
4. For the preparation of **7**, see Chapter 5 and also Hermkens, P.H.H.; Maarseveen, J.H.v.; Cobben, P.; Ottenheijm, H.C.J.; Kruse, C.G.; Scheeren, J.W. *Tetrahedron*, submitted.
5. Arx, E. von; Faupel, M.; Bruggen, M. *J.Chromatogr.* **1976**, *120*, 224.

Chapter 3.3

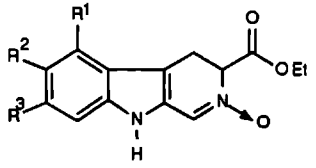
1,3-DIPOLAR CYCLOADDITION OF NITRONES WITH NITRILES. SCOPE AND MECHANISTIC STUDY.

The [3+2] cycloaddition reactions of nitriles with 1,3-dipoles containing an orthogonal double bond (nitrilium betaines, diazonium betaines) are well documented[1] and afford useful synthetic routes to a variety of five-membered heterocyclic ring systems. In contrast, relatively few examples of the cycloaddition of nitriles to 1,3-dipoles lacking a double bond (the class of azomethinium betaines) are known.[1] Recently we reported[2] that **1**, a nitrone derived from N-hydroxytryptophane as well as several other nitrones undergo cycloaddition to *geminal* dinitriles with complete regioselectivity to give Δ^4-1,2,4-oxadiazolines.

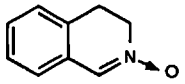
In general, the knowledge and understanding of the reaction scale of 1,3-dipoles and dipolarophiles are intimately connected with mechanistic questions. It is generally accepted that 1,3-dipolar cycloadditions of nitrones to alkenes are single-step, concerted, four-center reactions.[3] However, it has been suggested[1] that polarized dipolarophiles (*e.g.* nitriles) may undergo concerted, but not necessarily synchronous cycloadditions. We now report an extensive investigation of the scope as well as a mechanistic study of the nitrone-nitrile cycloaddition.

Synthetic Scope

The nitrones **1-6** were used as 1,3-dipoles in this investigation. The synthesis of nitrones **3** and **4** has



1: $R^1=R^2=R^3=H$
2: $R^1=R^2=H$, $R^3=OMe$
3: $R^1=Cl$, $R^2=R^3=H$
4: $R^1=R^3=H$, $R^2=Cl$



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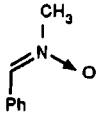
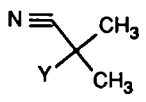


6

been described in chapter 3.2 and the nitrones **1**[2], **2**[2], **5**[4] and **6**[5] were prepared according to known procedures.

A survey of the nitriles used in this study is given in Table I. They are divided into three classes based on structural characteristics, *viz.*, 2-substituted-2-cyanopropanes (**7**), ylidenemalononitriles (**8**) and directly substituted cyano derivatives (**9**). The results of the cycloaddition reactions of the nitrones

Table I. Survey of nitriles divided into three classes.



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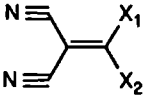

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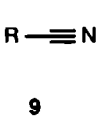

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	Y	Ref.		X_1	X_2	Ref.		R
a	NO_2	6	a	H	Ph	11	a	CCl_3
b	CN	7	b	H	p-Cl-C_6H_4	12	b	COOEt
c	COOEt	8	c	H	2-furanyl	11	c	$N(Me)_2$
d	Ph	9	d	Ph	Ph	11	d	Ph
e	Me	10	e	SMe	SMe	11	e	Me

1-4, 5 and **6** with the nitriles **7-9** to give the cycloadducts **10, 11** and **12**, respectively, are listed in Table II.

Reactivity scale of nitriles.

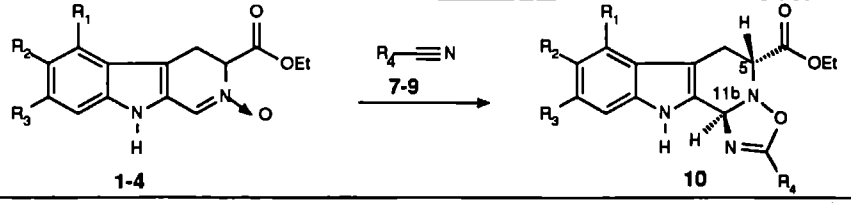
Within the series of reactions of **1** with nitriles **7-9** (entries 1-9 and 13-23) it is possible to study the reactivity scale of the nitriles. In the series of 2-substituted-2-cyanopropanes (**7**) (entries 1-8) we studied exclusively the inductive influence of the substituent on the reactivity of the nitrile function. Under thermal conditions the nitriles **7a** and **7b**[2] react smoothly to give the cycloadducts **10a** and **10b** in nearly quantitative yields (entries 1 and 2), whereas the nitriles **7c-e** react sluggishly giving **10c-e** in low yields (entries 3,5 and 7). The yield of the reaction leading to **10e** (entry 7) could be improved by the use of a ten-fold excess of the dipolarophile. Acceptable yields of **10c-e** were accomplished, when high pressure conditions (12 kbar) were used (entries 4, 6 and 8). It is apparent that the reactivity of the nitriles decreases with decreasing electron withdrawing ability of the substituent *e.g.* NO_2~CN > COOEt > C_6H_5~alkyl.

Monosubstituted ylidene malononitriles **8a-c** react easily with **1** (entries 9, 13 and 14). Remarkable is the higher reactivity of **8b** and **c** versus **8a**. The disubstituted ylidene malononitrile **8d** reacted very sluggishly under thermal conditions and gave cycloadduct **10l** in a low yield (entry 15). Reaction of the disubstituted ylidene malononitriles **8d** and **8e** under high pressure conditions however, solved this problem and in high yields the adducts **10l** and **10m** were isolated (entries 16 and 17).

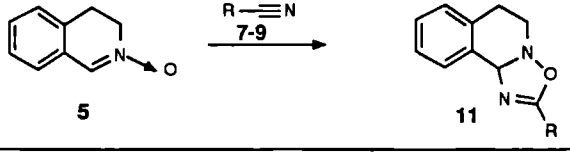
For the directly substituted cyano derivatives **9**, the reactivity decreases in the order **9a>9b>9c>9d** (entries 18-21). Nitrile **9e** did not react under thermal or under high pressure conditions (entries 22 and 23). These results demonstrate that both electron withdrawing and strongly electron donating groups facilitate the reaction.

Cycloaddition reactions of nitriles with nitrones **1-4** proceeded with complete stereoselectivity -*viz.* C(5) and C(11b) substituents have an *trans* orientation- which is similar to earlier observed cycloadditions of these nitrones.[2] This was judged on account of the chemical shift values of the H(5) and H(11b) protons, which are not very different[13] from analogous shift values of the corresponding

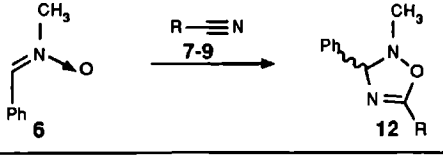
Table II. Reactions of nitrones (1-6) with nitriles (7-9) (1.5 equivalents) in toluene.



Entry	R_1	R_2	R_3	R_4	Reaction conditions	Product	Yield[a] (%)
1	H	H	H	$C(NO_2)Me_2$	80°C, 2h.	10a	97
2				$C(CN)Me_2$	80°C, 1.5h.	10b[b]	99
3				$C(COOEt)Me_2$	80°C, 7d.	10c	26
4					50°C, 12 kbar, 28h.[c]		75
5				$C(Ph)Me_2$	80°C, 7d.	10d	11
6					50°C, 12 kbar, 2d.[c]		56
7				$C(Me)_3$	80°C, 6d.	10e	12 (48)[d]
8					50°C, 12 kbar, 3d.[c]		87[d]
9	H	H	H	C(CN)=CHPh	80°C, 33 h.	10f[b]	78
10			OMe		60°C, 7h.	10g[b]	95
11		Cl	H		80°C, 7h.	10h	72
12		H	Cl		80°C, 7h.	10i	72
13	H	H	H	C(CN)=CH(p-C_6H_4Cl)	80°C, 3h.	10j	97
14				C(CN)=CH(2-C_4H_3O)	80°C, 1.5h.	10k	100
15				$C(CN)=C(Ph)_2$	80°C, 1d.	10l	11
16					RT, 12 kbar, 1d.[c]	10l	84
17				$C(CN)=C(SMe)_2$	RT, 12 kbar, 1d.[c]	10m	78
18				CCl_3	80°C, 2 minutes	10n	100
19				COOEt	80°C, 1.5h. (80°C, 0.5h.[d])	10o	85 (98)[d]
20				$N(Me)_2$	80°C, 20h., (80°C, 3.5h.[d])	10p	93 (100)[d]
21				Ph	80°C, 2d.	10q	87[d]
22				Me	refluxing acetonitrile	no reaction	
23					RT, 12 kbar, 3d.[c]	no reaction	



24	$C(CN)Me_2$	100°C, 1h.	11a	100[d]
25	CCl_3	100°C, 2 minutes	11b	100[d]
26	Ph	100°C. 5d.	11c	56[d]



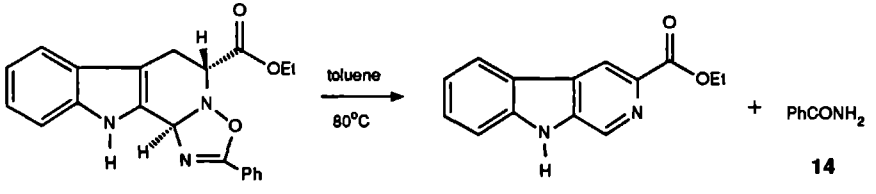
27	$C(CN)Me_2$	110°C, 10d.	12a[b]	85[d]
28	CCl_3	110°C, 2 minutes	12b	100[d]
29	Ph	110°C, 10d.	12c	57[d]

a) based on isolated products b) see reference 2 c) reaction in DMF d) ten-fold excess of dipolarophile

protons of the *trans* compounds **10b** and **10f** (see reference 2). The *trans* orientation of the C(5) and C(11b) substituents is ascribed to steric influence of the ethoxycarbonyl function in the transition state. Furthermore, in the case of the ylidenemalononitriles **8a-c**, only the less sterically hindered of the two diastereotopic nitrile functions adds to the 1,3-dipole, which is in agreement with earlier results.[2] Confirmation of the influence of steric hindrance was found in the low reactivity of the disubstituted ylidenemalononitriles **8d** and **e**.

As mentioned earlier[2], the β-carboline carboxyethylester **13** (β-CCE) was formed up to 10% yield when the cycloaddition reactions were allowed to stand for days under thermal conditions. We found that cycloaddition product **10q** partially decomposed to give **13** and benzamide (**14**) (Scheme I) when

Scheme I



10q **13** **14**

it was kept under cycloaddition reaction conditions for 2 days. This problem could be overcome by the use of a ten-fold excess of dipolarophile or high pressure conditions. (see Table II)

Reactivity scale of nitrones.

The nitrones **1-4** made it possible to study the influence of indole substitution on the reactivity of the 1,3-dipole in cycloaddition to nitriles. Reaction of nitrile **8a** with **1-4** (entries 9-12) clearly demonstrates that **2** is the most reactive nitrone as a result of the electron donating effect of the methoxy substituent.[2] The chloro substituents of **3** and **4** showed no clear effect on the reactivity with regard to the unsubstituted nitrone **1**. With regard to nitrones **5** and **6**, nitrone **1** is more reactive as shown by reaction of **5** and **6** with some nitriles (entries 24-29). With the highly reactive nitrile **9a** it is not possible to make a distinction between the reactivity of these nitrones. However, the less reactive nitriles **7b** and **9d** demonstrate that the order of reactivity is **1** > **5** > **6**, which is in agreement with our earlier observations.[2]

Kinetics

Pseudo-first-order reaction rates were determined in 2-methoxyethanol, by using nitrone **1** and a tenfold excess of the dipolarophile; the reaction was monitored with a dilatometer.[14] The results are presented in Table III.

We studied the reactivity of **7b** and ethyl crotonate in order to compare the nitrile cycloaddition with the well-known analogous cycloaddition of alkenes (Table 3, entries 1 and 2). Only a small difference in rate ($k_{ethyl\ crotonate}$ / $k_{dimethylmalononitrile}$=2.7) was found. Extrapolation for the k_2-value of ethyl

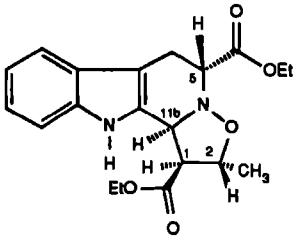
Table III. Dilatometric k_2-values for cycloadditions of 1 with dipolarophiles in 2-methoxyethanol.

Entry	dipolarophile	10^5k2 (l/mol.sec) 85°C	90°C	100°C	110°C	Product
1	dimethylmalononitrile (7b)			553		10b
2	ethyl crotonate	564		1517[a]		15
3	p-nitrobenzonitrile	21.2				10r
4	p-cyanobenzaldehyde	9.20				10s
5	p-cyanobenzoic acid	6.28				10t
6	p-chlorobenzonitrile	5.35				10u
7	benzonitrile	3.02	4.48	8.52	21.10	10q
8	p-tolunitrile	2.60[b]			17.60	10v
9	anisonitrile	2.03[b]			14.00	10w

a) Extrapolation from 85°C to 100°C, with correction factor 2.7 (Huisgen, see ref. 3b)

b) Extrapolation from 110°C to 85°C, isoentropic ($\Delta S^{\ddagger}$=-23.6 cal/mol deg)

crotonate from 85°C to 100°C was done *isoentropically*.[15] Noteworthy is the complete regio- and stereoselectivity of the cycloaddition of ethyl crotonate with **1** to give isoxazolidine **15**. Structure



15

assignment was made in analogy with other cycloadditions of **1**,[16] *viz.*, the C(5) and C(11b) substituents have an *trans*-orientation (*vide-supra*), electron withdrawing substituents on C(1) favours *endo*-[17] and alkyl substituents on C(2) *exo*-orientation. The proton coupling constant $J_{11b,1}$=9.9 Hz also supports the relative stereochemistry as depicted in **15**.[17,18]

The reaction of *para*-substituted benzonitriles was studied in order to determine a Hammett plot (Table III , entries 3-9). The reactivity differences made it necessary to determine the reaction rates at two temperatures. The more reactive nitriles (entries 3-7) were measured at 85°C, and the remaining nitriles (entries 8 and 9) at 110°C. Extrapolation of the k2-values of the nitriles of entries 8 and 9 from 110°C to 85°C occurred *isoentropically*. The necessary activation parameters are obtained from benzonitrile, *i.e.* entry 7 ($\Delta S^{\ddagger}$=-23.6 cal/mol.deg and $\Delta H^{\ddagger}$=20.1 kcal/mol). The Hammett plot showed an excellent linear correlation between the log k-values and the substituent constants (σ-values)[19], *viz.*, ρ=0.96 (corr.coeff. = 0.995) (Figure I).

Because of the insolubility of nitrone **1** in most solvents we studied the influence of solvent polarity upon the reaction rate with the better soluble though less reactive **5**. The reaction of **5** with dimethylmalononitrile (**7b**) was studied at 100°C in three solvents covering a wide range of E_T values[20] (Table IV). We observed a notoriously small effect of solvent polarity. In agreement with Huisgen's[3b] study of the cycloaddition of nitrones with alkenes we found an inverse influence of

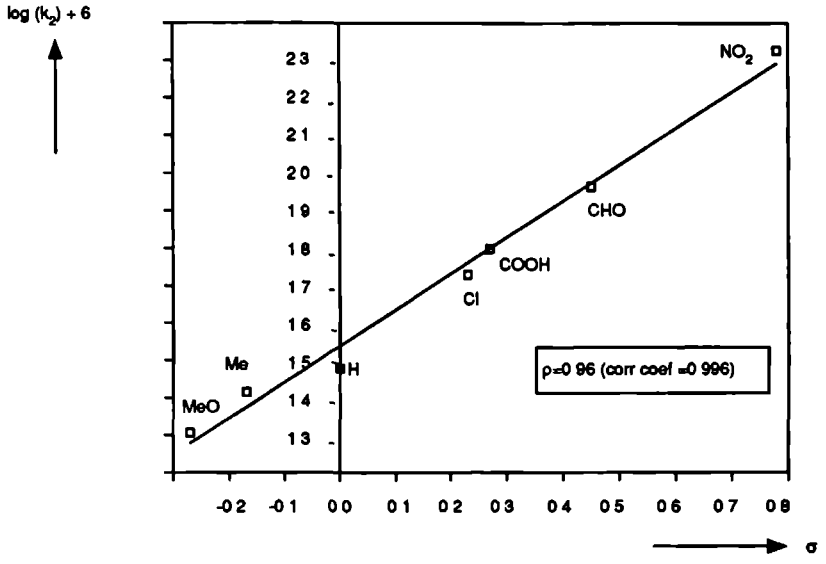


Figure I. Hammett Plot

Table IV. Rates in various solvents for reaction of **5** with **7b** at 100°C

solvent	10^3k_2 l/mol sec.	ET kcal/mol
toluene	15.1	33.9
phenetole	11.5	36 4
2-methoxyethanol	1.44 (1.38)[a]	52.3

a) normal second-order experiment

solvent polarity ($k_{toluene}/k_{2\text{-methoxyethanol}}$=10.5).

Discussion

The regioselective formation of the Δ^4-1,2,4-oxadiazoline ring in the cycloaddition of nitriles **7-9** with nitrones **1-6**, can be rationalized by using Frontier Molecular Orbital (FMO) theory.[21] In our previous report[2] we suggested that the regioselectivity of the reaction depends on a favourable HOMO(nitrone)-LUMO(nitrile) interaction. This would imply that the reactivity will increase if *nitrones* become more electron-rich and *nitriles* more electron-deficient.[2] The results of the study at hand are in line with this assumption.

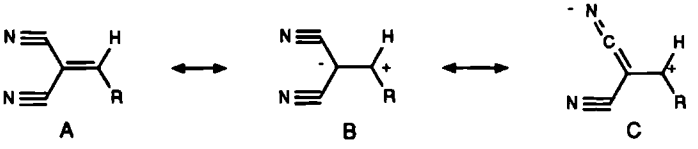
A measure for the electron richness of conjugated nitrones is the weighted average of the first two ionization potentials. The values for **1**[2,16], **5**[22] and **6**[22] are 8.40, 8.46 and 8.56 eV, respectively. This is in agreement with the reactivity order **1**>**5**>**6**. The enhanced reactivity of **2** as compared with that of **1** suggests an average IP-value lower than 8.40 eV. This must be the result of the electron donating effect of the methoxy substituent.

In the case of 2-substituted-2-cyanopropanes **7** and directly substituted cyano derivatives **9** we found

an increas in reactivity of the nitrile function with increasing electron withdrawing ability of the substituent, *viz.*, the reaction order for **7**; NO_2~CN>COOEt>C_6H_5~CH_3 and that for **9**; CCl_3>COOEt>C_6H_5.

In the 1,3-dipolar cycloadditions with ylidenemalononitriles **8** the nitrones add selectively to the nitrile function[23], whereas the reactivity still strongly depends on the nature of the β-substituent. The influence of β-substituents has already been demonstrated in [2+2][24] cycloadditions of the alkene moiety of **8**. Generally, electron withdrawing substituents will decrease the conjugate resonance stabilization (Scheme II) and consequently make the alkene function more reactive. Although the

Scheme II



nitrile function is further away from the β-substituent, we observed a similar, and even stronger substituent effect. This may be attributed to the diminished contribution of resonance structure C in the order 2-furanyl<<4-Cl-C_6H_4<<C_6H_5.

The reactivity of the nitrile **9c** with the strongly electron donating dimethylamino group is the result of a crossover in the frontier orbital control, so that the regiochemistry will now depend on a favourable HOMO(nitrile)-LUMO(nitrone) interaction. This is in agreement with the classification by Sustmann[25] that nitrone cycloadditions are type II processes, in which both HOMO-LUMO interactions contribute to the stabilization of the transition-state. A U-shaped reactivity curve of dipolarophiles is a necessary consequence. In both HOMO-LUMO interactions, overlap of orbitals with comparable terminal coefficients, *i.e.* orbitals of the nitrogen and carbon atoms of the nitrile with the carbon and oxygen of the nitrone, respectively will lead to the Δ^4-1,2,4-oxadiazoline ring.

The inverse influence of the solvent polarity is an indication of a slight decrease of the polarity in the *transition state* (TS) versus the polarity of the reactants. This supports the concerted nature of this 1,3-dipolar cycloaddition and is in agreement with an early TS.

The term *concerted* does not necessarily imply that the two new σ-bonds are developed in the TS to precisely the same extent. If in the TS of cycloaddition of nitrones with a polarized dipolarophile -such as the nitrile- the making of one bond lays behind the closure of the other σ-bond, partial charges at the centers of the weak incipient bond can be stabilized. Therefore, a Hammett plot of the cycloaddition of nitrone **1** with p-substituted benzonitriles has been determined. The excellent linear correlation between the log k-values and substituent constants (σ-values)[19] shows that all the p-substituted benzonitriles are far away from the crossover in the U-shape reactivity curve. The observed valu for ρ=0.96 is slightly higher than the analogous value found by Huisgen[3b] (ρ=0.77) in the cycloaddition of the nitrone **6** with p-subtituted styrenes. This higher ρ-value is not necessarily a result of a more polar transition state for the nitrile cycloadditions. It may also be a reflection of a

smaller energy gap between the HOMO(nitrone) and LUMO(nitrile) compared to the HOMO(nitrone) and LUMO(alkene).[26]

In conclusion, the results of this study are consistent with Huisgen's[3b] kinetic study of the 1,3-dipolar cycloaddition of nitrones with alkenes. Consequently, it can be concluded that nitrone-nitrile cycloadditions should not be considered mechanistically different from nitrone-alkene cycloadditions.

Experimental Section

Melting points were taken on a Koefler hot stage (Leitz-Wetzlar) and are uncorrected. Ultraviolet spectra were measured with a Perkin-Elmer spectrometer, Model Lambda 5. Proton magnetic resonance spectra were measured on a Bruker WH-90 spectrometer. Chemical shifts are reported as δ-values relative to tetramethylsilane as an internal standard; deuteriochloroform was used as solvent unless stated otherwise. Mass spectra were obtained using a double-focusing VG 7070E spectrometer. Thin-layer chromatography (TLC) was carried out using silica gel F-254 plates (thickness 0.25 mm). Spots were visualized with a UV hand lamp, iodine vapor, or Cl_2-TDM.[27] For high-performance liquid column chromatography (Jobin Yvon) Merck silica gel H (type 60) was used. Solvent systems used are, A: ($MeOH/CHCl_3$, 1/99, v/v), B: ($MeOH/CHCl_3$, 3/97, v/v), C:($MeOH/CHCl_3$, 5/95, v/v), D: ($MeOH/CHCl_3$, 7/93, v/v)

General Procedure Cycloadditions.

Thermal Reaction Conditions. A stirred solution of nitrone (0.5 mmol) and nitrile (0.75 mmol) in dry toluene (10 mL) was allowed to react under the conditions given in Table II. The reaction was monitored by TLC. The mixture was then concentrated *in vacuo* and the resultant residue subjected to column chromatography to yield compounds **10**, **11** or **12**(Table II). Recrystallization was always accomplished from CH_2Cl_2 / MeOH / n-hexane. Spectroscopic data for these derivatives are recorded in Table V.

High-Pressure Reaction Conditions. The nitrone (0.5 mmol) and the nitrile (0.75 mmol) were dissolved in dry DMF (1.5 mL) and brought into a Teflon high-pressure vessel, which was placed in a high-pressure apparatus. Pressure was raised to 12 kbar and to the appropriate temperature (see Table II). The mixture was then concentrated *in vacuo* and the resultant residue subjected to column chromatography to yield compounds **10c**, **d**, **e**, **l**, and **m** (Table II). Recrystallization was always accomplished from CH_2Cl_2 / MeOH / n-hexane. Spectroscopic data for these derivatives are recorded in Table V.

Decomposition of cycloadduct 10q

A solution of **10q** (361 mg, 1.0 mmol) in dry toluene (15 mL) was kept at 80°C for two days under argon atmosphere. After evaporation of the solvent the residue was subjected to flash chromatography (silica gel 60H, eluens $CHCl_3$/MeOH, 99/1, v/v) to give 310 mg (86%) of **10q**, 34 mg (14%) of **13** and 18 mg (14%) of benzamide (**14**).

Compound 20: Spectroscopical data are identical with earlier published results.[16]

Kinetic Experiments.

*Equipment:*The dilatometer was made according to a literature method.[28] The important features of this design are: a) The coil had a capacity of about 20 ml. b) The precision capillary was 25 cm long and its inner diameter was 0.35 mm. A microprocessor controlled constant temperature bath Tamson TMV 70, filled with ethylene glycol (70 L) was used. Temperature fluctuations in the bath were less than 0.004°C.

*Execution:*Although all the reactions obeyed the second-order rate law, pseudo-first-order reaction rates were determined, by using a tenfold excess of dipolarophile.[14] The concentration of nitrone was in all the experiments 0.116M. Further, standard procedure was followed.[3b] Rate constants were calculated on a IBM XT personal computer, using a non-linear regression fit program based on the Gauss method.[29] Second-order rate constants were reproducible within 5%.

Table V. Spectroscopic data of cycloadducts **10**, **11**, **12** and **15**.

prod	mp (°C)	Rf (Solv Sys.)	UV(MeOH) λ max (nm)	IR ν(cm^{-1})	Mass Spectrum	^{1}H NMR δ (ppm)
10a[a]	176-181	0 62 (A)	222, 277	1732 (C=O) 1675 (C=N) 1556 (NO_2)	EIMS (70 eV) m/z 372 (M^+, 21%), 258 ([$C_{14}H_{14}N_2O_3$]$^+$, 25%), 185 ([$C_{11}H_{10}N_2$]$^+$, 100%), 169 (59%)	6 36 (br s, 1H, NH), 7 52-7 02 (m, 4H, C(7)-C(10)H), 6 19 (s, 1H, C(11b)H), 4 31 and 4 29 (2q, 2H,diastereotopic protons, OCH_2CH_3), 3 93 (X part of ABX spectrum, 1H, ^{3}J 3 6 Hz, ^{3}J 11 8 Hz, C(5)H), 3 20 and 3 01 (AB part of ABX spectrum, 2H, ^{2}J 15 6 Hz, ^{3}J 3 6 Hz, ^{3}J 11 8 Hz, C(6)H_2), 1 88 (s, 6H, 2xCH_3), 1 32(t, 3H, OCH_2CH_3)
10c[a]	141-144	0 55 (A)	222, 274	1740 (C=O) 1665 (C=N)	CIMS (100 eV) m/z 400 ([M+1]$^+$, 12%), 243 ([$C_{14}H_{14}N_2O_2$+1]$^+$, 22%), 142 ([$C_7H_{11}NO_2$+1]$^+$, 73%), 114 ([$C_6H_{10}O_2$]$^+$, 100%)	8 98 (br s, 1H, NH), 7 49-7 00 (m, 4H, C(7)-C(10)H), 6 14 (s, 1H, C(11b)H), 4 31 (q, 2H, OCH_2CH_3),4 16 and 4 14 (2q, 2H, OCH_2CH_3), 3 88 (Xpart of ABX spectrum, 1H, ^{3}J 3 9 Hz, ^{3}J 12 3 Hz, C(5)H), 3 17 and 3 00 (AB part of ABX spectrum, 2H, ^{2}J 15 4 Hz, ^{3}J 3 9 Hz, ^{3}J 12 3 Hz, C(6)H_2), 1 50 (s, 6H, 2xCH_3), 1 34 (t, 3H, OCH_2CH_3), 1 23 (t, 3H, OCH_2CH_3)
10d	oil	0 53 (A)	220, 272 280 (sh) 289 (sh)	3400 (NH) 1740 (C=O) 1660 (C=N)	EIMS (70 eV) m/z 403 (M^+, 7%), 330 ([M-COOEt]$^+$, 2%), 258 ([$C_{14}H_{14}N_2O_3$]$^+$ 68%), 185 ([$C_{11}H_{10}N_2O$]$^+$, 86%), 71 (100%)	8 80 (br s, 1H, NH), 7 46-6 96 (m, 9H, C(7)-C(10)H and Ph), 6 11 (s, 1H, C(11b)H), 4 17 (q, 2H, OCH_2CH_3), 3 61 (X part of ABX spectrum, 1H, ^{3}J 2 6 Hz, ^{3}J 12 3 Hz, C(5)H), 3 08 and 2,95 (AB part of ABX spectrum, 2H, ^{2}J 15 6 Hz, ^{3}J 2 6 Hz, ^{3}J 12.3 Hz, C(6)H_2), 1 61 (s, 6H, 2xCH_3), 1 21 (t, 3H, OCH_2CH_3)
10e[a]	169-172	0 78 (B)	220, 272 280 (sh) 289 (sh)	3370 (NH) 1735 (C=O) 1660 (C=N)	EIMS (70 eV) m/z 341 (M^+, 12%), 284 ([$C_{15}H_{14}N_3O_3$]$^+$, 22%), 258 ([$C_{14}H_{14}N_2O_3$]$^+$, 40%), 241 ([$C_{14}H_{13}N_2O_2$]$^+$, 11%), 185 ([$C_{11}H_{10}N_2O$]$^+$, 100%), 169 ([$C_{11}H_{10}N_2$]$^+$, 38%)	8 93 (br s, 1H, NH), 7 52-7 00 (m, 4H, C(7)-C(10)H), 6 10 (s, 1H, C(11b)H), 4 36 and 4 34 (2q, 2H, OCH_2CH_3), 3 76 (X part of ABX spectrum, 1H, ^{3}J 3 3 Hz, ^{3}J 12 0 Hz, C(5)H), 3 15 and 3 04 (AB part of ABX spectrum, 2H, ^{2}J 15 5 Hz, ^{3}J 3 3 Hz, ^{3}J 12 0 Hz, C(6)H_2), 1 36(t, 3H, OCH_2CH_3), 1 25 (s, 9H, C(CH_3)$_3$)
10h[a]	172-174	0 66 (A)	225, 300, 306	2236 (nitrile) 1730 (C=O) 1640 (C=N)	EIMS (70 eV) m/z 448 ([M+2]$^+$,0 9%), 446 (M^+,1 6%), 294 ([$C_{14}H_{13}ClN_2O_3$]$^+$, 5%), 292 ([$C_{14}H_{13}ClN_2O_3$]$^+$, 14%), 204 ([$C_{11}H_7ClN_2$]$^+$, 46%), 202 ([$C_{11}H_7ClN_2$]$^+$ 100%)	9 03(br s, 1H, NH), 7 98-7 01 (m, 9H, C(8)-C(10)H, CHPh, Ph), 6 46 (s, 1H, C(11b)H), 4 40 (q, 2H, OCH_2CH_3), 3 92 (X part of ABX spectrum, 1H, ^{3}J 3 7 Hz, ^{3}J 11 2 Hz, C(5)H), 3 72 and 3 27 (AB part of ABX spectrum, 2H, ^{2}J 15 7 Hz, ^{3}J 3 7 Hz, ^{3}J 11 2 Hz, C(6)H_2), 1 36 (t, 3H, OCH_2CH_3)
10l[a]	179-182	0 67 (A)	228, 303	2230 (nitrile) 1730 (C=O) 1640 (C=N)	EIMS (70 eV) m/z 448 ([M+2]$^+$, 0 1%), 446 (M^+, 0 3%), 294 ([$C_{14}H_{13}ClN_2O_3$]$^+$, 2%), 292 (6%), 276 ([$C_{14}H_{11}ClN_2O_2$]$^+$, 8%), 274(21%), 204 ([$C_{11}H_7ClN_2$]$^+$, 35%), 202 (100%)	8 80 (br s, 1H, NH), 7 98-7 82 (m, 3H, C(9)C(10)H and C=CH-Ph), 7 53-7 11 (m, 6H, C(7)H and Ph) 6 41 (s, 1H, C(11b)H), 4 37 (q, 2H, OCH_2CH_3), 3 89 (X part of ABX spectrum, 1H, ^{3}J 2 7 Hz, ^{3}J 11 1 Hz C(5)H), 3 17 and 3 04 (AB part of ABX spectrum, 2H, ^{2}J 16 4 Hz, ^{3}J 2 7 Hz , ^{3}J 11 1 Hz, C(6)H_2), 1 36 (t, 3H, OCH_2CH_3)
10j[a]	177-180	0 73 (A)	221, 293(sh) 318	2238 (nitrile) 1717 (C=O) 1642 (C=N)	FABMS (7 kV at 1 4 mA) m/z 449 ([M+3]$^+$, 2%), 447 ([M+1]$^+$, 4%), 243 ([$C_{14}H_{14}N_2O_2$+1]$^+$, 100%)	8 40 (br s, 1H, NH), 7 91-7 11 (m, 9H, C(7)-C(10)H and C=CH-, and C_6H_4Cl), 6 36 (s, 1H, C(11b)H), 4 36 (q, 2H, OCH_2CH_3), 3 88 (X part of ABX spectrum, 1H, ^{3}J 4 0 Hz, ^{3}J 9 9 Hz, C(5)H), 3 23 and 3 06 (AB part of ABX spectrum, 2H, ^{2}J 15 1 Hz, ^{3}J 4 0 Hz, ^{3}J 9 9 Hz, C(6)H_2), 1 37 (t, 3H, OCH_2CH_3)

a) Satisfactory micro analysis were obtained for these compounds (C±0 5%, H±0 2%, N±0 4%)

Table V. Spectroscopic data of cycloadducts **10**, **11**, **12** and **15**.

Prod	mp (°C)	Rf (Solv Sys)	UV (MeOH) λmax (nm)	IR ν(cm^{-1})	Mass Spectrum	^{1}H NMR δ (ppm)
10k[a]	167-171	0 51 (B)	222, 282, 345	2235 (nitrile) 1730 (C=O) 1650 (C=N)	EIMS (70 eV) m/z 402 (M^+, 1 5%), 258 ([$C_{14}H_{14}N_2O_3$]$^+$, 35%), 144 ([$C_8H_4N_2O$]$^+$, 100%)	200 MHz 8 54 (s, 1H, NH), 7 75 (d, 1H,C(α')H), 7 63 (s, 1H, =CH-), 7 50 (d, 1H, C(7)H), 7 40 (d, 1H, C(10)H, 7 35 (d, 1H, C(β)H), 7 24 and 7 14 (2xm, 2H, C(8) and C(9)H), 6 67 (dd, 1H,C(β')H), 6 38 (s, 1H, C(11b)H), 4 39 and 4 36 (2q, 2H, OCH_2CH_3), 3 86 (X part of ABX spectrum, 1H,^{3}J 3 0 Hz, ^{3}J 11 1 Hz, C(5)H), 3 22 and 3 06 (AB part of ABX spectrum, 2H, ^{2}J 15 9 Hz, ^{3}J 3 0 Hz, ^{3}J 11 1 Hz, C(6)H_2), 1 38 (t, 3H, OCH_2CH_3)
10l[a]	148-152	0 29 (A)	209, 221 273(sh), 282, 290, 319	2220 (nitrile) 1742 (C=O) 1655 (C=N)	EIMS (70 eV) m/z 488 (M^+, 1%), 258 ([$C_{14}H_{14}N_2O_3$]$^+$, 11%), 240 ([$C_{14}H_{12}N_2O_2$]$^+$, 38%), 168 (100%)	8 69 (br s, 1H, NH), 7 55-7 07 (m, 14H, C(7)-C(10)H and 2xPh), 6 06 (s, 1H, C(11b)H), 4 18 (q, 2H, OCH_2CH_3), 3 80 (X part of ABX spectrum, 1H, ^{3}J 3 8 Hz, ^{3}J 10 9 Hz,C(5)H) 3 18 and 2 94 (AB part of ABX spectrum, 2H, ^{2}J 15 5 Hz, ^{3}J 3 8 Hz, ^{3}J 10 9 Hz, C(6)H_2), 1.29 (t, 3H, OCH_2CH_3)
10m[a]	143-146	0 72 (B)	222, 283 339	2220 (nitrile) 1710 (C=O) 1620 (C=N)	EIMS (70 eV) m/z 428 (M^+, 7%), 355 ([M-COOEt]$^+$, 12%), 258 ([$C_{14}H_{14}N_2O_3$]$^+$, 23%), 240 ([$C_{14}H_{12}N_2O_2$]$^+$, 35%), 185 (34%), 170 ([$C_6H_6N_2S_2$], 100%), 169 (56%)	8 46 (br s, 1H, NH), 7 53-7 04 (m, 4H, C(7)-C(10)H), 6 32 (s, 1H, C(11b), 4 35 (q, 2H, OCH_2CH_3), 3 92 (X part of ABX spectrum, 1H, ^{3}J 3 7 Hz, ^{3}J 11 6 Hz, C(5)H), 3 20 and 3 05 (AB part of ABX spectrum, 2H, ^{2}J 15 7 Hz, ^{3}J 3,7 Hz, ^{3}J 11 6 Hz, C(6)H_2, 2 69 (s, 3H SCH_3), 2 57 (s, 3H, SCH_3), 1 30 (t, 3H, OCH_2CH_3)
10n[a]	159-162	0 75 (B)	209 (sh) 223, 273 280 (sh) 289 (sh)	3359 (NH) 1720 (C=O) 1666 (C=N)	EIMS (70 eV) m/z 405 ([M+4]$^+$, 1 2%), 403 ([M+2]$^+$, 3 3%), 401 (M^+, 3 5%), 284 ([$C_{15}H_{14}N_3O_3$]$^+$, 13%), 240 (7%), 185 (11%), 168 (43%), 108 (97%), 44 (100%)	8 46 (br s, 1H, NH), 7 56-7 04 (m, 4H, C(7), C(10)H, 6 44 (s, 1H, C(11b(H), 4 36 and 4 34 (2q, 2H, OCH_2CH_3), 4 01 (X part of ABX spectrum, 1H, ^{2}J 3 6 Hz, ^{3}J 11 0 Hz, C(5)H), 3 26 and 3,06 (AB part of ABX spectrum, 2H, ^{2}J 15 4 Hz, ^{3}J 3 6 Hz, ^{3}J 11 0 Hz, C(6)H_2), 1 35 (t, 3H, OCH_2CH_3)
10o[a]	84-88	0 67 (B)	204 (sh) 220, 272 280 (sh) 289 (sh)	3320 (NH) 1745 (C=O) 1708 (C=O) 1654 (C=N)	EIMS (70 eV) m/z 357 (M^+, 1%), 284 ([$C_{15}H_{14}N_3O_3$]$^+$, 2%), 240 ([$C_{14}H_{12}N_2O_2$]$^+$, 2%), 185 ([$C_{11}H_{10}N_2O$]$^+$, 5%), 169 ([$C_{11}H_9N_2$]$^+$, 16%), 54 (100%)	8 63 (br s, 1H, NH), 7 53-7 02 (m, 4H, C(7)-C(10)H), 6 40 (s, 1H, C(11b)H), 4 38 (q, 2H, OCH_2CH_3), 4 33 (q, 2H, OCH_2CH_3), 3 95 (X part of ABX spectrum, 1H, ^{3}J 6 6 Hz, ^{3}J 8 1 Hz, C(5)H), 3 24 and 3 03 (AB part of ABX spectrum, 2H, ^{2}J 15 4 Hz, ^{3}J 6 6 Hz, ^{3}J 8 1 Hz, C(6)H_2), 1 33 (2xt, 6h, 2xOCH_2CH_3)
10p[a]	73-77	0 38 (B)	208 (sh) 223, 273 280 289 (sh) 318	1738 (C=O) 1665 (C=N)	EIMS (70 eV) m/z 328 (M^+, 65%), 284 ([$C_{15}H_{16}N_4O_2$]$^+$, 100%), 258 ([$C_{14}H_{14}N_2O_3$]$^+$, 15%), 210 ([$C_{12}H_8N_2O_2$]$^+$, 95%), 185 (42%), 168 (30%)	9 71 (br s, 1H, NH), 7 61-6 96 (m, 4H, C(7)-C(10)H), 6 21 (s, 1H, C(11b)H), 4 33 (q, 2H, OCH_2CH_3), 4 07)X part of ABX spectrum, 1H, C(5)H), 3 47-2 90 (AB part of ABX spectrum, 2H, C(6)H_2), 2.90 (s, 6H, $N(CH_3)_2$), 1.36 (t, 3H, OCH_2CH_3)
10q[a]	174-176	0 69 (A)	207(sh) 222, 269, 273(sh), 281(sh) 290(sh)	3290 (NH) 1734 (C=O) 1652 (C=N)	EIMS (70 eV) m/z 361 (M^+, 6%), 288 ([$C_{18}H_{14}N_3O$]$^+$, 5%), 258 ([$C_{14}H_{14}N_2O_3$]$^+$, 10%), 240 ([$C_{14}H_{12}N_2O_2$]$^+$, 7%), 185 (21%), 168 (38%), 103 ([C_7H_5N]$^+$, 100%)	8 81(br s, 1H, NH), 7 96-7 85 (m, 2H, C(2)-Ph-ortho-H), 7 54-7 04 (m, 7H, C(7)-C(10)H and C(2)Ph-meta,para-H), 6 38 (s, 1H, C(11b)H), 4 40 (q, 2H, OCH_2CH_3), 3 94 (X part of ABX spectrum, 1H, ^{3}J 2 5 Hz, ^{3}J 11 8 Hz, C(5)H), 3 22 and 3 11 (AB part of ABX spectrum, 2H, ^{2}J 15 5 Hz, ^{3}J 2 5 Hz, ^{3}J 11 8 Hz, C(6)H_2), 1 40 (t, 3H, OCH_2CH_3)

a) Satisfactory micro analysis for these compounds were obtained (C±0.5%, H±0.2%, N±0.4%).

Table V. Spectroscopic data of cycloadducts **10, 11, 12** and **15**.

product	mp (°C)	Rf (Solv Sys)	UV (MeOH) λmax(nm)	IR ν (cm^{-1})	Mass Spectrum	^{1}H NMR δ(ppm)
10r[a]	168-172	0 32 (A)	222 276	1730 (C=O) 1650 (C=N) 1354 (NO_2)	EIMS (70 eV) m/z 406 (M^+, 1%), 333 ([$C_{18}H_{13}N_4O_3$]$^+$, 1%), 258 ([$C_{14}H_{14}N_2O_3$]$^+$, 8%), 240 ([$C_{14}H_{12}N_2O_2$]$^+$, 12%), 185 (16%), 168 ([$C_{11}H_8N_2$]$^+$, 81%), 148 ([$C_7H_4N_2O_2$]$^+$, 100%)	8 23 and 8 02 (AB spectrum, 4H, ^{3}J 9 4 Hz, $C_6H_4NO_2$), 7 57-7 09 (m, 4H, C(7)-C(10)H), 6 41 (s, 1H, C(11b)H), 4 40 (q, 2H, OCH_2CH_3), 3 91 (X part of ABX spectrum, 1H, ^{3}J 2 9 Hz, ^{3}J 11 9 Hz, C(5)H, 3 23 and 3 11 (AB part of ABX spectrum, 2H, ^{2}J 15 6 Hz, ^{3}J 2 9 Hz, ^{3}J 11 9 Hz, C(6)H_2), 1 38 (t, 3H, OCH_2CH_3)
10s[a]	165-169	0 49 (A)	220, 267 280 (sh) 289 (sh)	3260 (NH) 1742 (C=O) 1700 (C=O) 1650 (C=N)	EIMS (70 eV) m/z 389 (M^+, 1%), 316 ([$C_{19}H_{14}N_3O_2$]$^+$, 1%), 258 (20%), 240 (31%), 185 (42%), 169 (100%), 131 ([C_8H_5NO]$^+$, 58%)	10 06 (s, 1H, CHO), 8 74 (br s, 1H, NH), 8 05 and 7 93 (AB spectrum, 4H, ^{3}J 8,4 Hz, C_6H_4CHO), 7 56-7 00 (m, 4H, C(7-)C(10)H), 6 40 (s, 1H, C(11b)H), 4 39 (q, 2H, OCH_2CH_3), 3,92 (X part of ABX spectrum, 1H, ^{3}J 2 5 Hz, ^{3}J 12 8 Hz, C(5)H), 3 21 and 3 11 (AB part of ABX spectrum, 2H, ^{2}J 15 7 Hz, ^{3}J 2 5 Hz, ^{3}J 12.8 Hz, C(6)H_2), 1 39 (t, 3H, OCH_2CH_3)
10t[a,b]	167-170	0 71 (B)	220, 253 282 (sh) 289,(sh)	3370 (NH) 1731 (C=O) 1715 (C=O) 1653 (C=N)	EIMS (70 eV) m/z 419 (M^+, 1%), 346 ([M-COOEt]$^+$, 1%), 258 (7%), 240 (13%), 161 ([$C_9H_7NO_2$]$^+$, 100%)	8 60 (br s, 1H, NH), 8 12 and 8 00 (AB spectrum, 4H, ^{3}J 9 2 Hz, C_6H_4-$COOCH_3$), 7 59-7 09 (m, 4H, C(7)-C(10)H), 6 40 (s, 1H, C(11b)H), 4 41 (q, 2H, OCH_2CH_3), 3 95 (s, 3H, OCH_3), 3 93 (X part of ABX spectrum, 1H, ^{3}J 2 5 Hz, ^{3}J 13 1 Hz, C(5)H), 3 23 and 3 13 (AB part of ABX spectrum, 2H, ^{2}J 15 5 Hz, ^{3}J 2 5 Hz, ^{3}J 13 1 Hz, C(6)H_2), 1 35 (t, 3H, OCH_2CH_3)
10u[a]	164-167	0 78 (A)	219 249	3395 (NH) 1750 (C=O) 1645 (C=N)	EIMS (70 eV) m/z 397 ([M+2]$^+$, 3%), 395 (M^+, 9%), 324 (M+2-COOEt]$^+$, 5%), 322 ([M-COOEt]$^+$, 14%), 258([$C_{14}H_{14}N_2O_3$]$^+$, 21%), 240 ([$C_{14}H_{12}N_2O_2$]$^+$, 14%), 185 ([$C_{11}H_{10}N_2O$]$^+$, 44%), 168 (100%)	8 62 (br s, 1H, NH), 7 87-7 03 (m, 8H, C(7)C(10)H and C_6H_4Cl), 6 35 (s, 1H, C(11b)H), 4 40 (q, 2H, OCH_2CH_3), 3 91 (X part of ABX spectrum, 1H, ^{3}J 3 0 Hz, ^{3}J 12 0 Hz, C(5)H) 3 22 and 3 10 (AB part of ABX part of ABX spectrum, 2H, ^{2}J 15 6 Hz, ^{3}J 3 0 Hz, ^{3}J 12 0 Hz, C(6)H_2) 1 38 (t, 3H, OCH_2CH_3)
10v[a]	164-168	0 42 (A)	222 247 267	1738 (C=O) 1658 (C=N)	EIMS (70 eV) m/z 375 ([M^+, 8%), 302 ([M-COOEt]$^+$, 8%), 258 ([$C_{14}H_{14}N_2O_3$]$^+$, 24%), 169 ([$C_{11}H_{10}N_2$]$^+$, 29%), 117 (100%)	8 86 (br s, 1H, NH), 7 84-7 04 (m, 8H, C(7)C(10) and C_6H_4-CH_3), 6 36 (s, 1H, C(11b)H), 4 39 (q, 2H, OCH_3CH_2) 3,91 (X part of ABX spectrum, 1H, ^{3}J 1 9 Hz, ^{3}J 12 4 Hz, C(5) H), 3 19 and 3 09 (AB part of ABX spectrum, 2H, ^{2}J 15 1 Hz, ^{3}J 1 9 Hz, ^{3}J 12 4 Hz, C(6) H_2), 2 38 (s, 3H, C_6H_4-CH_3), 1 39 (t, OCH_3CH_2)
10w[a]	178-182	(A)	216 270	1733 (C=O) 1657 (C=N)	EIMS (70 eV) m/z 391 (M^+, 9%), 318 ([M-COOEt]$^+$, 7%), 258 ([$C_{14}H_{14}N_2O_3$]$^+$, 36%), 185 ([$C_{11}H_{10}N_2O$]$^+$, 61%), 169 ([$C_{11}H_{10}N_2$]$^+$, 44%), 133 ([C_8H_7NO]$^+$, 100%)	9 27 (br s, 1H, NH), 7 84 and 6 89 (AB spectrum, 4H, ^{3}J 9 0 Hz, C(2)-C_6H_4-OCH_3), 7 55-7 02 (m, 4H, C(7)C10)H), 6 37 (s, 1H, C(11b)H), 4 40 (q, 2H, OCH_2CH_3), 3 93 (Xpart of ABX spectrum, ^{3}J 2 4 Hz, ^{3}J 12 4 Hz, C(5)H), 3 81 (s, 3H, C_6H_4-OCH_3), 3 21 and 3 11 (AB part of ABX spectrum, 2H, ^{2}J 15 6 Hz, ^{3}J 2 4 Hz, ^{3}J 12 4 Hz, C(6)H_2), 1 39 (t, 3H, OCH_2CH_3)
11a	oil	0 75 (B)	214 258 (sh) 264, 271 286, 307 (sh)	2240 (nitrile) 1669 (C=N)	EIMS (70 eV) m/z 241 (M^+, 4%), 173 ([$C_{10}H_9N_2$]$^+$, 14%), 147 ([C_9H_9NO]$^+$, 100%)	7 55-7 03 (m, 4H, C(7)-C(10)H), 5 96 (s, 1H, C(10b)H), 3 48-2 63 (m, 4H, C(5)-C(6)H_2), 1 67 (s, 6H, 2xCH_3)

a) Satisfactory micro analysis were obtained for these compounds (C±0.5%, H±0 2%, N±0.4%) b) Esterfied to methoxycarbonyl with thionyl chloride in methanol.

Table V. Spectroscopic data of cycloadducts **10, 11, 12** and **15**

product	mp (°C)	Rf (Solv Sys.)	UV (MeOH) λmax(nm)	IR ν (cm^{-1})	Mass Spectrum	^{1}H NMR δ (ppm)
11b	oil	0 86 (B)	214, 256 (sh), 263, 270, 286 307 (sh)	1660 (C=N)	EIMS (70 eV) m/z 296 ([M+6]$^+$, 0 3%), 294 ([M+4]$^+$, 2 8%), ([M+2]$^+$, 8 4%), 290 (M$^+$, 8 4%), 173 ([$C_{10}H_9N_2O$]$^+$, 20%), 147 ([C_9H_9NO]$^+$, 100%)	7 61-7 06 (m, 4H, C(7)-C(10)H), 6 20 (s, 1H, C(10b)H), 3 60-2 67 (m, 4H, C(5)-C(6)H_2)
11c	oil	0 82 (B)	205 237, 263 (sh), 271 (sh)	1645 (C=N)	EIMS (70 eV) m/z 250 (M$^+$, 6%), 173 ([$C_{10}H_9N_2O$]$^+$, 10%), 147 ([C_9H_8NO]$^+$, 100%), 77 (58%)	8 02-7 90 (m, 2H, C(2)Ph-ortho-H's), 7 68-7 07 (m, 7H, C(7)-C(10)H and C(2)Ph-meta, para-H), 6 13 (s, 1H, C(10b)H), 3 74-2 67 (m, 4H, C(5)-C(6)H_2)
12b	oil	0 90 (B)	204 238	1662 (C=N)	EIMS (70 eV) m/z 284 ([M+6]$^+$, 0 3%), 282 ([M+4]$^+$, 3 1%), 280 ([M+2]$^+$, 9 2%) , 278 (M$^+$, 9 6%), 161 ([$C_9H_9N_2O$]$^+$, 5%), 134 ([C_8H_8NO]$^+$, 100%), 77 (33%)	7 34 (s, 5H, Ph), 5 73 (s, 1H, C(3)H), 2 97 (s, 3H, N-CH_3)
12c	oil	0 76 (B)	204 238	1660 (C=N)	EIMS (70 eV) m/z 238 (M$^+$, 24%), 193 ([$C_{14}H_{11}N$]$^+$, 5%), 135 ([C_8H_9NO]$^+$, 100%), 77 ([C_6H_5]$^+$, 60%)	8 04-7 93 (m, 2H, C(5)-Ph-ortho-H's), 7 50-7 24 (m, 8H, C(3)-Ph and C(5)-Ph-meta,para-H's), 5 77 (s, 1H, C(3)H), 2 96 (s, 3H, N-CH_3)
15[a]	187-189	0 26 (B)	222, 273 280 290 (sh)	3200 (NH) 1740 (C=O) 1721 (C=O)	EIMS (70 eV) m/z 372 (M$^+$, 39%), 299 ([M-COOEt]$^+$, 38%), 185 ([$C_{11}H_{10}N_2O$]$^+$, 100%), 169 ([$C_{11}H_9N_2$]$^+$, 42%)	8 03 (br s, 1H, NH), 7 48-6 98 (m, 4H, C(7)-C(10)H), 5 11 (d, 1H, ^{3}J=9 9 Hz, C(11b)H), 4 93 and 4 85 (2xq, 1H, ^{3}J 7 3 Hz, ^{3}J 8 0 Hz, C(2)H), 4 43 (X part of ABX spectrum, 1H, ^{3}J 6 6 Hz, ^{3}J 6 8 Hz, C(5)H), 4 27 (q, 2H, OCH_2CH_3), 3 83 (q, 2H, OCH_2CH_3), 3 76 (2xd, 1H, ^{3}J 7 3 Hz and ^{3}J 9 9 Hz, C(1)H), 3 61 and 3 52 (AB part of ABX spectrum, 2H , ^{2}J 15 3 Hz, ^{3}J 6 6 Hz, ^{3}J 6 8 Hz, C(6)H_2), 1 35 (d, 3H, ^{3}J 8 0 Hz, C(2)CH_3), 1 33 (t, 3H , OCH_2CH_3), 0 87 (t, 3H, OCH_2CH_3)

a) Satisfactory micro analysis were obtained for these compounds (C±0 5%, H±0.2%, N±0.4%).

References and Notes

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14. According to Huisgen, second-order rates were calculated from pseudo-first-order rates as follows: $k_2=k_1\psi$ / ([dipolarophile]-0.5[nitrone]); see reference 3b.
15. It is assumed that the activation entropy $\Delta S^{\ddagger}$ is constant and for ethyl crotonate in reaction with nitrones it is established to be $\Delta S^{\ddagger}$=-28 cal/mol.deg; see reference 3b.
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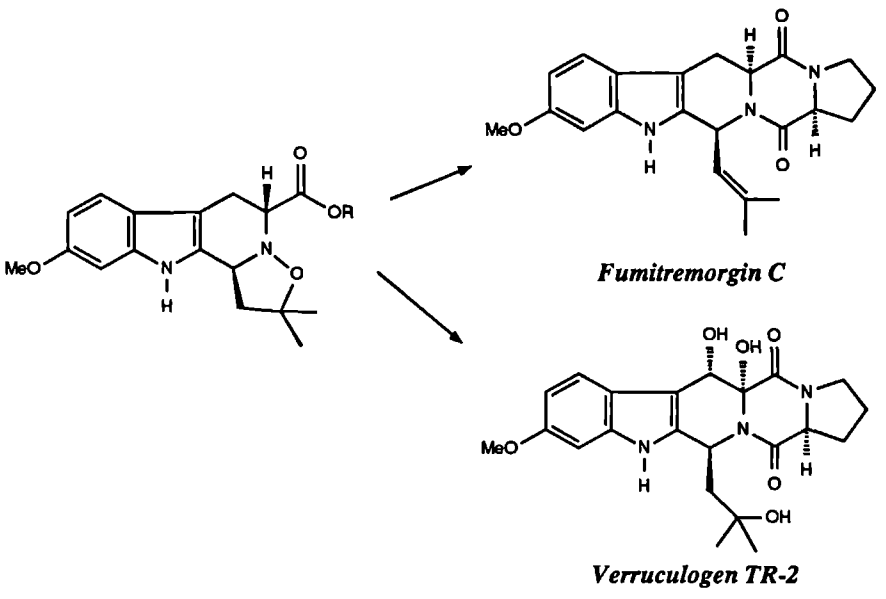
CHAPTER 4

Application of an Isoxazolidine in the Stereoselective Synthesis of Fumitremorgin C and Verruculogen TR-2



Fumitremorgin C

Verruculogen TR-2

"First Total Synthesis of Fumitremorgin C", Pedro H.H. Hermkens, Ralf Plate,Harrie C.J. Ottenheijm, *Tetrahedron* (**1988**), *44*, 1991.

"First Total Synthesis of Verruculogen TR-2", Pedro H.H. Hermkens, Ralf Plate, Harrie C.J. Ottenheijm, *Tetrahedron Letters* (**1988**), *29*, 1323.

"Stereoselective synthesis of verruculogen TR-2. A new oxidation method to dehydro-β-carbolines.", Pedro H.H. Hermkens, Chris G. Kruse, Hans W. Scheeren, Harrie C.J. Ottenheijm, *J.Org.Chem.*, submitted.

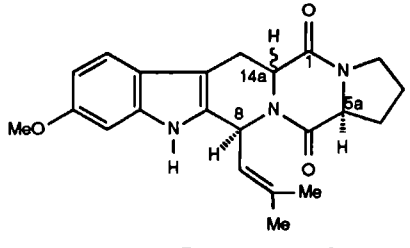
Chapter 4.1

INTRODUCTION

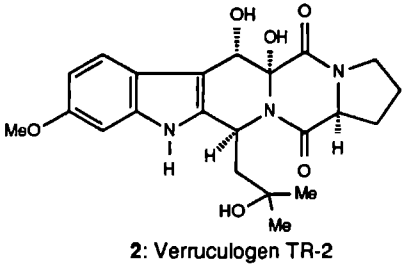
Increased research on mycotoxins has led to the discovery of fungal metabolites that induced neurological manifestations in vertebrate animals that include sustained or intermittent tremors.[1-12] Fungi capable of producing tremorgic metabolites can be found on a variety of important agricultural commodities. The fungal tremorgens can be classified into six groups based on chemical relationship.[9] The compounds of one of these groups-the fumitremorgin-verruculogen group- are biochemically derived from tryptophan, proline and one or more mevalonic acid moieties.[6] Seven members of this group are at the moment isolated and identified, including in most cases their stereochemistry (three members are given in Scheme I). In efforts to determine the mode of action of fungal tremorgens, it has become apparent thet they provide usefull tools in the study of central nervous system functions. In general, they interfere in the mechanisms resposible for the release of CNS neurotransmitters.[13-18] Although particular molecular features responsible for the tremorgenic activity in the fumitremorgin-verruculogen group have not been completely identified, there are indications that the conformation and configuration of the dioxopiperazine moiety affects this tremorgenic activity[16].

We became interested in the fumitremorgins as attractive synthetic targets because of their biological activity. The first target we settled upon was fumitremorgin C (**1**)[2,3,6,8,9] a mycotoxin isolated from

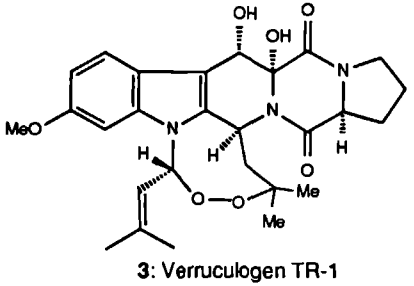
Scheme I



1: Fumitremorgin C



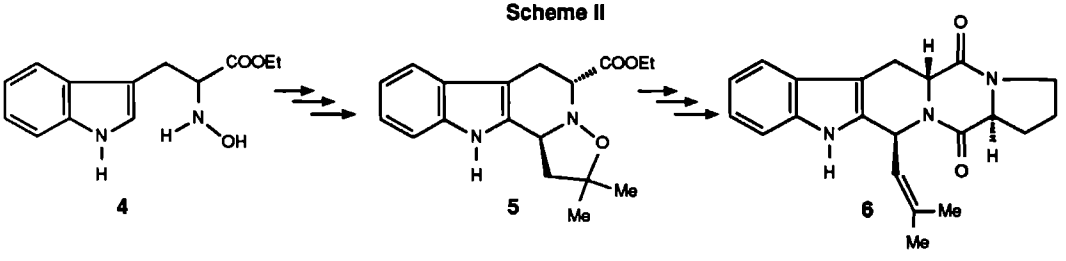
2: Verruculogen TR-2



3: Verruculogen TR-1

Aspergillus Fumigatus. An earlier approach[19] *via* an isoxazolidine (*e.g.* **5**) -derived from N-hydroxytryptophan (**4**)- demonstrated that the this was an efficient route to the skeleton of the fumitremorgins *i.e.* **6** (Scheme II). A further study of this route is discussed in chapter 4.2, which

Scheme II



eventually resulted in the total synthesis of fumitremorgin C.

The second target was the more functionalized verruculogen TR-2 (**2**)[3,6,8,9,11], a mycotoxin initially isolated from *Aspergillus Fumigatus* (Chapter 4.3).

Over the last years other members of the fumitremorgin-verruculogen class of mycotoxins have been targets for total synthesis.[20]

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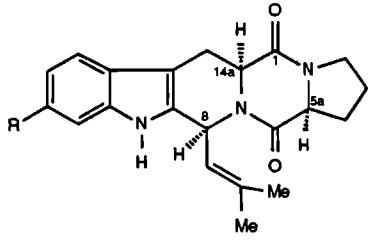
Chapter 4.2

TOTAL SYNTHESIS OF (-)-FUMITREMORGIN C.

The structure of fumitremorgin C, as first reported in 1977,[1a] contains three chiral carbon atoms. The absolute configuration at C(5a) and C(8) is as depicted in formula **1a**.[1] The stereochemistry at C(14a) has not been ascertained[2]. Thus, the total synthesis of fumitremorgin C is desirable -amongst others- to confirm the assigned structure and to determine the precise stereochemistry.

Recently, we reported the first stereoselective approach to tetrahydro-β-carbolines (c.f. **7**) having a masked C(1) 2-hydroxy-2-methylpropyl side chain moiety.[3] Subsequent to the development of this efficient, N-hydroxytryptophan mediated synthesis of these tetrahydro-β-carbolines, one of our goals has been the synthesis of the skeleton of fumitremorgins.[4] Through the synthesis of the fumitremorgin C analog **2b**, we became evidence -though inconclusive- that this compound is not the skeleton of fumitremorgin C (**1a**) but a C(14a) epimer.



1a Fumitremorgin C
1b
2a,b (C(14a)-epimer)
3a,b (C(8)-epimer)
4a,b (C(8) and C(14a)-epimer)

a: R=OCH_3
b: R=H

Unfortunately, our approach to **2b** employing the cycloadduct **7b** invariably led to compounds having a *trans*-relationship between the tetrahydro-β-carbolines C(1) and C(3) substituents (Scheme I). So the problem we faced was a selective epimerisation at the carbon atom C(3) of **7** or one of its successors.

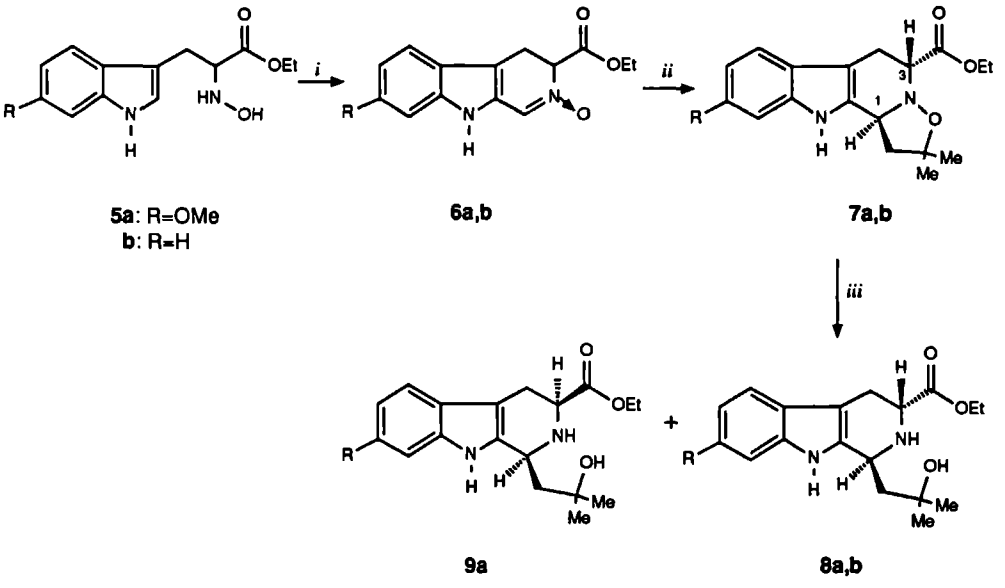
In this chapter a straight-forward synthesis of the optically pure tremogenic mycotoxin fumitremorgin C (**1a**) and it isomers **2a-4a** is presented.

Results

Nitrone **6a** (Scheme I) was prepared by a known procedure[3,4,5] from **5a**. 1,3-Dipolar cycloaddition of isobutene with **6a** proceeded regio- and stereoselectively to give quantitatively the *trans*-adduct **7a**. The desired cleavage of the isoxazolidine N-O bond was accomplished by treatment of **7a** with zinc dust in acetic acid.[6]

Surprisingly[7], this procedure gave a diastereomeric mixture of **8a** (*trans*) and **9a** (*cis*) in 98% yield.

Scheme I



5a: R=OMe
b: R=H

6a,b **7a,b**

9a **8a,b**

i) $HC(OMe)_3$ / H^+ ii) isobutene in toluene (120°C, 8 bar) iii) Zn / HOAc

The ratio **8a/9a** varied from 12.5/1 to 25/1. We have not established unambiguously whether this epimerisation at C(3) occurs with **7a** or with **8a**, mainly because the efficiency of this process was too low to be of synthetic value. We rather preferred to study the epimerisation of **8a** and its progeners.

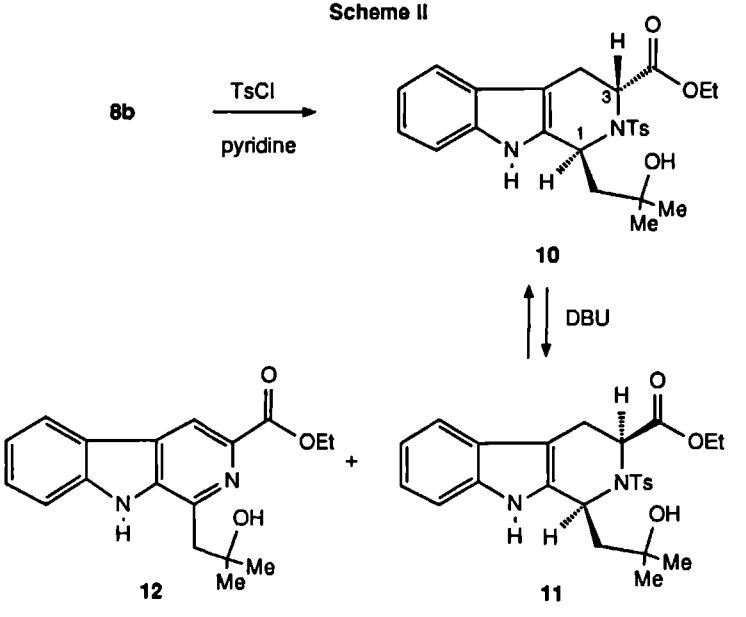
Epimerisation

In our hands the isoxazolidine tetrahydro-β-carbolines **7** could not be epimerised. Treatment with base (NaH or tBuOK in dimethoxyethane) gave **12** in 75% yield. (Scheme II) Therefore we selected the N(2) amides as target molecules for epimerisation studies. As a model compound we selected the N(2) sulfonamide derivative **10**, which was prepared in 82% yield by treatment of **8b**[2] with TosCl in pyridine.

An efficient method for epimerisation of **10** was only found after several unsuccesful attempts. No isomerisation occured when **10** was treated with Et_3N in dichloromethane. Employment of the stronger base NaOEt in ethanol led to the undesired β-carboline **12** as the main product.[8]The method of choice for the anticipated epimerisation appeared to be treatment of **10** with DBU in dichloromethane at room temperature. The *cis/trans* equilibrium ratio of this reaction was estimated as **11/10**=8.5/1.0. Minute quantities (5%) of **12** as side product were observed.[9]

The epimerisation occurred selectively at the C(3) carbon since reaction of **10** with DBU in a mixture of CD_3OD and dichloromethane led to deuterium incorporation at the C(3) carbon exclusively.

Scheme II



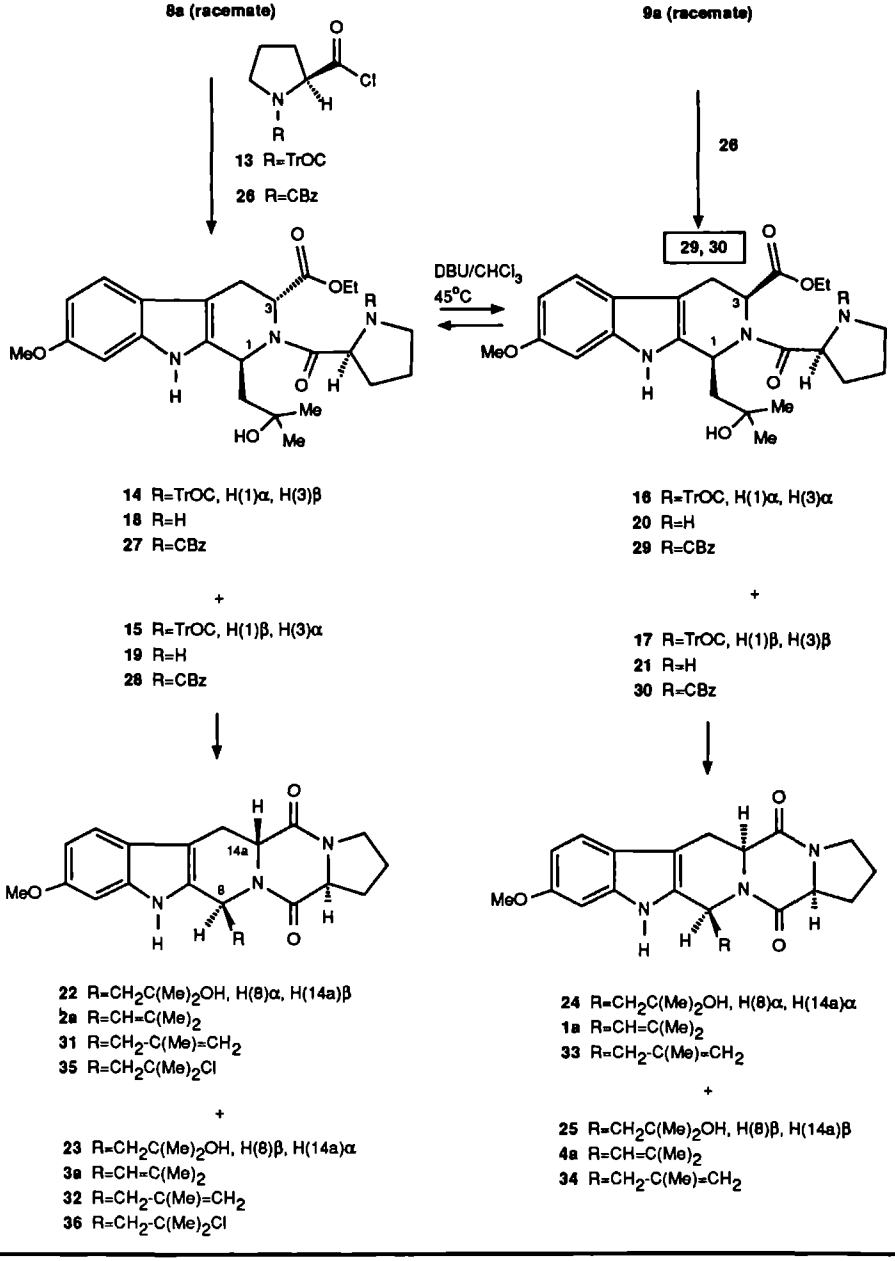
Encouraged by these results we selected the diastereomeric amides **14** and **15** (Scheme III) as targets for epimerisation. These compounds were prepared in the following manner. Treatment of the racemic amine **8a** with the acid chloride of TrOC-L-proline (**13**) at -20°C[10] in the presence of triethylamine provided in 77% yield the amides **14** and **15** (diastereomeric ratio 1/1.5), which were separated by column chromatography. When the experiment was carried out without triethylamine a complete kinetic resolution was observed leading to the exclusive formation of diastereomer **15**. Subsequent addition of the base resulted in formation of **14** from the residual **8a** and **13**.

Isomerisation of the key intermediate **14** into its epimer **16** was achieved as follows. When **14** was treated with DBU in chloroform for 2 days at 45°C an equilibrium ratio of **14/16**=1/1 was observed. Fortunately, this procedure afforded no oxidized β-carbolines. Subsequently, **15** was treated in the same manner to give an equilibrium ratio of **15/17**=2/3. These diastereomeric mixtures could easily be separated by column chromatography where upon the *trans* compounds were subjected again to this epimerisation proces. In this way the *trans* compounds **14** and **15** could be converted nearly completely into the corresponding *cis* compounds **16** and **17**, respectively.

The proline amine functions of the compounds **14-17** were deprotected by means of zinc dust in refluxing methanol to give the amines **18-21** which -under these conditions- cyclized to give the pentacyclic compounds **22-25**. We noticed that the *trans* compounds **22** and **23** were obtained quantitatively within 10 minutes, whereas the *cis* compounds **24** and **25** were obtained in 63% and 45% yield, respectively only after prolonged reaction of 3 days.

Comparison of CPK models revealed that in **24** and **25** there is considerable steric hindrance

Scheme III



between the side-chain and the proline moiety. This observation might rationalise the rather sluggish dioxopiperazine formation in the *cis*-series.

Alternatively, the four pentacycles **22-25** could be prepared -though less efficiently in the case of **24**

and **25**- from the isomers **8a** and **9a** (*vide supra*). Thus coupling of **8a** and **9a** with CBz-L-proline acid chloride (**26**) (Scheme III) provided the amides **27** and **28** (ratio 1/1.25) in 79% and **29** and **30** (ratio 1/1.15) in 71% yield, respectively. Again kinetic resolution was observed. Chromatographic separation of the diastereomers, followed by deprotection by catalytic hydrogenation and subsequent ring closure of the intermediates **18-21** afforded again the pentacyclic dioxopiperazines **22** (100%), **23** (100%), **24** (10%) and **25** (10%), respectively. The yields again are indicating that ring closure of the *cis* compounds is less favourable.

That no racemisation had taken place in the proline moiety during the coupling procedure was secured with the chiral shift reagens tris[3-((heptafluoropropyl)hydroxymethylene)camphorato]-europium (III) as described earlier.[4] Stereochemical structure assignments are made by extending our previous results[4] assuming that no deviant behaviour is caused by the methoxy moiety on the indole nucleus.

For obvious reasons we finally subjected the pentacyclic compounds **22** and **23** to DBU in $CHCl_3$ at 60°C (*vide supra*) as well as to NaOEt in ethanol at 60°C. However, no epimerisation was observed.

Finally, transformation of the alcohol functions of **22-25** into the alkene functions of **1a-4a** was accomplished by means of $SOCl_2$ in pyridine at -40°C.[2] The desired alkenes **1a-4a** were accompanied by products due to Hofmann eliminations and -in some cases- by the corresponding chlorides. Thus **24** gave **1a** (4%) and **33** (44%) and **22** gave **2a** (65%), **31** (11%) and **35** (7%) and **23** gave **3a** (28%), **32** (13%) and **36** (12%) and **25** gave **4a** (5%) and **34** (16%). It was discouraging to observe that in the *cis* series the dehydration afforded mainly the undesired products of Hofmann elimination. A tentative rationale comes from studying the CPK models; the proton participating in the desired Saytzeff elimination is sterically more hindered than the corresponding proton in the *trans* series. Attempts to isomerize the olefins **31-34** into **1a-4a** by means of catalytic amounts of acid (H_2SO_4 or TFA) or a metal complex ($PdCl_2$, $RhCl(PPh_3)_3$ failed.[11] Upon completion of this synthesis of fumitremorgic C, Nakagawa *et.al*[12] reported that isomerization of comparable olefins was possible employing the transition metal catalyst $Fe_3(CO)_{12}$.

Of the four pentacycles **1a-4a** only the product **1a** possessed spectral characteristics identical to those reported for fumitremorgin C.[4] This comparison completed the first total synthesis of this natural product.[13] From this result we concluded that the product **2b** we reported earlier[4] is indeed the C(14a) epimeric analog of the natural product.

Discussion

The synthesis of fumitremorgin C (**1a**) as well as the developed method to achieve the desired tetrahydro-β-carboline having a *cis* relationship of the C(1), C(3) substituents demonstrates the utility of N-hydroxytryptophan in the synthesis of indole alkaloids.

Epimerisation at the C(3) carbon in 1,3-disubstituted-tetrahydro-β-carbolines was accomplished only when N(2) amides were used and steric interactions absent.

A draw-back of the synthetic route was that in the *cis* series the dehydration afforded mainly the undesired products of a Hofmann elimination. Nakagawa *et.al*[13] demonstrated later on that a better

position in the reaction sequence for this elimination reaction is in the stage before ring closure (*e.g.* compounds **16** and **17**).

Experimental Section

Melting points were taken on Koefler hot stage (Leitz-Wetzlar) and are uncorrected. Ultraviolet spectra were measured with a LKB spectrophotometer, Model 4050. Proton magnetic resonance spectra were measured on a Bruker WH-90 spectrometer. Chemical shifts are reported as δ values (parts per million) relative to tetramethylsilane as an internal standard. As a chiral reagent we used tris[3-((heptafluoropropyl) hydroxy-methylene)-*d*-camphorato]europium(III) (Janssen Chimica, Belgium). Mass spectra were obtained using a double focusing VG 7070E spectrometer. Thin layer chromatography (TLC) was carried out using Merck precoated silicagel F-254 plates (thickness, 0.25 mm). Spots were visualized with a UV hand lamp, iodine vapor, Cl_2-TDM.[14] For column chromatography Merk silicagel 60H was used. Solvent systems used were as follows: system A: $CHCl_3$/MeOH,93/7,v/v; system B: $CHCl_3$/MeOH,97/3, v/v; system C:EtOAc; system D:$CHCl_3$/MeOH,99/1,v/v.

2,2-Dimethyl-5-(ethoxycarbonyl)-9-methoxy-4,5,6,11b-tetrahydro-isoxazolidin[2,3-a]-β-carboline (7a)

Thermal reaction conditions: A stirred solution of **6a**[5] (2.04 g, 7.06 mmol) in dry toluene (75 mL) and isobutene (50 mL) was heated for 4h at 120°C in a 250 mL pressure vessel. The pressure increased up to 9 bar. Filtration of the mixture gave 1.99 g (82%) product. Evaporation of the filtrate and recrystallisation of the residue (CH_2Cl_2) gave additional 0.3 g (12%) of **7a**. Total yield (94%); mp 263-265°C ; R_f 0.57 (solvent system A); UV (methanol) λmax 226,265, 269, 297 nm, λmin 250, 280 nm; EIMS (70 eV) m/z (relative intensity), 344 (M^+, 73%), 271 $[C_{16}H_{19}N_2O_2]^+$, 100%), 198 ($[C_{12}H_{10}N_2O]^+$, 55%), exact mass for $C_{19}H_{24}N_2O_4$ calcd. 344.1736, found 344.1731; ^1H NMR δ 7.89 (br s, 1H, NH), 7.37-6.70 (m, 2H, C(7)-C(8)H), 6.81 (s, 1H, C(10)H), 4.85 (X part of ABX spectrum, 1H, C(11b)H), 4.29 and 4.24 (2 q from diastereotopic protons, 2H, OCH_2CH_3), 4.09 (t, 1H, ^3J=7.0 Hz, C(5)H), 3.78 (s, 3H, OCH_3), 3.02 (d_2 2H, ^3J=7.0 Hz, C(6)H), 2.42 and 2.23 (AB part of ABX spectrum, 2H, ^2J=12.3 Hz, ^3J=6.7 Hz, ^3J=11.1 Hz, C(1)H_2), 1.42 and 1.31 (2xs, 6H, 2x CH_3), 1.28 (t, 3H, OCH_2CH_3); Anal. Calcd. for $C_{19}H_{24}N_2O_4$ (MW 344.412) : C, 66.26; H, 7.02; N, 8.13. Found: C, 66.11; H, 7.02; N, 8.12.

High-Pressure reaction conditions: The nitrone **6a** (288 mg, 1 mmol) and isobutene (3 mL) were dissolved in 4 mL DMF and brought into a teflon high-pressure vessel, which was placed in a high-pressure apparatus. After 20h at 12 kbar the reaction was completed as monitored by TLC. Filtration of the mixture gave 255 mg (75%) product. Evaporation of the filtrate and recrystallisation of the residue gave additional 40 mg (11%) of **7a**. Total yield 86%.

1-(2'-Hydroxy-methylpropyl)-3-(ethoxycarbonyl)-7-methoxy-1,2,3,4-tetrahydro-β-carboline (*trans* 8a, *cis* 9a)

Activated zinc dust was added portionwise to a stirred solution of **7a** (750 mg, 2.2 mmol) in 100 mL glacial acetic acid. The reaction mixture was kept at 40°C for 7 h and during that time argon was bubbled through the solution. The reaction was monitored by TLC (solvent system A). The reaction mixture was filtered and washed with CH_2Cl_2, the filtrate concentrated to dryness and the residue dissolved in CH_2Cl_2. This solution was washed successively with saturated $NaHCO_3$, brine, and dried over Na_2SO_4. The solvent was evaporated in vacuo and the residue subjected to flash chromatography ($CHCl_3$/MeOH, 98/2. v/v) to yield 32 mg (4%) of **9a** and 706 mg (94%) of **8a**. These products resisted crystallisation attempts.

cis-product 9a: R_f 0.30 (solvent system B); EIMS (70 eV) m/z (relative intensity) 346 (M^+, 20%), 273 ($[C_{16}H_{21}N_2O_2]^+$, 100%), 199 ($[C_{12}H_{11}N_2O]^+$, 25%); exact mass for $C_{19}H_{26}N_2O_4$ calcd. 346.1893, found 346.1892; ^1H NMR δ 8.86 (br s, 1H, N(9)H), 7.38-6.72 (m, 2H, C(5)-C(6)H), 6.81 (s, 1H, C(8)H), 4.44-4.08 (X part of ABX spectrum, 1H, C(1)H), 4.26 (q, 2H, OCH_2CH_3), 3.83 (s, 3H. OCH_3), 3.78 (X part of ABX spectrum, 1H, C(3)H), 3.04 and 2.77 (AB part of ABX spectrum, 2H, ^2J=15.3 Hz, ^3J=4.0 Hz, ^3J=11.5 Hz, C(4)H_2), 2.29 (br s, 2H, N(2)H and OH), 2.11 and 1.84 (AB part of ABX spectrum, 2H, ^2J=14.7 Hz, ^3J=6.0 Hz, ^3J=6.0 Hz, C(1)-CH_2), 1.39 (s, 6H, 2xCH_3), 1.33 (t, 3H, OCH_2CH_3).

trans-product 8a: R_f 0.27 (solvent system B); EIMS (70 eV) m/z (relative intensity) 346 (M^+, 39%),

273 (100%), 199 (40%); exact mass for $C_{19}H_{26}N_2O_4$ calcd. 346.1893, found 346.1889; ^{1}H NMR δ 7.83 (br s, 1H, N(9)H), 7.40-6.73 (m, 2H, C(5)-C(6)H), 6.82 (s, 1H, C(8)H), 4.51 (X part of ABX spectrum, 1H, C(1)H), 4.23 (q, 2H, OCH_2CH_3), 3.93 (X part of ABX spectrum, 1H, C(3)H), 3.83 (s, 3H, OCH_3), 3.10 and 2.74 (AB part of ABX spectrum, 2H, ^{2}J=15.6 Hz, ^{3}J=4.8 Hz, ^{3}J=10.0 Hz, C(4)H_2), 1.95 and 1.72 (AB part of ABX spectrum, 2H, ^{2}J=14.5 Hz, ^{3}J=5.1 Hz, ^{3}J=12.3 Hz, C(1)-CH_2), 1.40 and 1.25 (2xs, 6H, 2xCH_3), 1.31 (t, 3H, OCH_2CH_3).

1-(2'-Hydroxy-methylpropyl)-2-(tosyl)-3-(ethoxycarbonyl)-7-methoxy-1,2,3,4-tetrahydro--β-carboline (10)

To a stirring solution of **8b**[2] (1.24 g, 4 mmol) in dry pyridine (10 mL) at 0°C in an argon atmosphere was added dropwise tosyl chloride (840 mg, 4.4 mmol) in dry pyridine (5 mL). The solution was allowed to warm to room temperature. The reaction was completed within one hour. After dilution with CH_2Cl_2 (100 mL) the solution was succesively washed with, 2N aqueous HCl, brine and dried with Na_2SO_4. Evaporation of the filtrate gave 1.52 g (82%) of **10**, which was crystallized from CCl_4/Et_2O; mp 165-168°C; R_f 0.75 (solvent system A); UV (methanol) λmax 220, 264, 269, 275, 281, 287 nm, λmin 257 nm; CIMS (100 eV) m/z (relative intensity) 471 ($[M+1]^+$, 14%), 453 ($[C_{25}H_{29}N_2O_4S]^+$, 60%), 413 ($[C_{22}H_{25}N_2O_4S]^+$, 100%), 397 ($[C_{22}H_{25}N_2O_3S]^+$, 51%), 315 ($[C_{18}H_{23}N_2O_3]^+$, 35%); exact mass for $C_{25}H_{31}N_2O_5S$ calcd. 471.195, found 471.193; ^{1}H NMR δ 8.54 (br s, 1H, N(9)H), 7.80-7.00 (m, 8H, C(5)-C(8)H and C_6H_4), 5.20 (t, 1H, ^{3}J=6.1 Hz, C(1)H), 4.40 (q, 2H, OCH_2CH_3), 4.33 (X part of ABX spectrum, 1H, C(3)H), 3.20 and 2.97 (AB part of ABX spectrum, 2H, ^{2}J=16.2 Hz, ^{3}J=12.0 Hz, ^{3}J=4.4 Hz, C(4)H_2), 2.58 (br s, 1H, OH), 2.22 (s, 3H, p-CH_3), 2.08 (d, 2H, ^{3}J=6.1 Hz, C(1)-CH_2), 1.42 (t, 3H, OCH_2CH_3), 1.26 (s, 6H, 2xCH_3); Anal. Calcd. for $C_{25}H_{30}N_2O_5S$ (MW 470.589): C, 63.81; H, 6.43; N, 5.95. Found: C, 63.19; H, 6.52; N, 5.99.

Epimerisation of N(2)-sulfonamide 10

A solution of sulfonamide **10** (108 mg, 0.23 mmol) and DBU (35 mg, 0.23 mmol) in dry CH_2Cl_2 (10 mL) was stirred at room temperature in an argon atmosphere for 24 h. The reaction mixture was washed with 0.1N aqueous HCl and brine, and dried with Na_2SO_4. Evaporation of the solvent and subsequent flash chromatography (n-hexane/EtOAc, 75/25, v/v) of the residue gave the epimerized product **11** (85%), starting material (10%) and aromatised product **12** (5%).[2]

Compound 11: This product resisted crystallisation attempts.R_f 0.90 (solvent system A); EIMS (70 eV) m/z (relative intensity) 470 (M^+, 35%), 397 (100%), 169 (90%); exact mass for $C_{25}H_{30}N_2O_5S$ calcd. 470.1875, found 470.1878; ^{1}H NMR δ 9.98 (br s, 1H, N(9)H), 7.78-7.00 (m, 8H, C(5)-C(8)H and C_6H_4), 5.22 (br d, 2H, C(1)H and C(3)H), 4.05 and 4.03 (2q from diastereotopic protons, 2H, OCH_2CH_3), 3.44-3.28 (A part of ABX spectrum, 1H, C(4)Ha), 2.73-2.18 (m, 3H, C(4)Hb and C(1)-CH_2), 2.33 (s, 3H, p-CH_3), 2.09 (br s, 1H, OH), 1.61 and 1.36 (2xs, 6H, 2xCH_3), 1.16 (t, 3H, OCH_2CH_3).

Deuterium incorporation at C(3)-position of 10.

A solution of **10** (106 mg, 0.2 mmol) and DBU in CH_2Cl_2/CD_3OD, 8/2, v/v (4 mL) was stirred at room temperature in an argon atmosphere for 24 h. The solvent was evaporated and the residue subjected to flash chromatography ($CHCl_3$) to yield 140 mg (80%) C(3)-deuterated **11**: EIMS (70 eV) m/z (relative intensity) 471 (M^+, 22%), 398 ($[C_{22}H_{24}DN_2O_3S]^+$, 100%), 170 ($[C_{11}H_8DN_2]^+$, 69%); ^{1}H NMR δ 9.96 (br s, 1H, N(9)H), 7.72-6.98 (m, 8H, C(5)-C(8)H and C_6H_4), 5.19 (br d, 1H, C(1)H), 4.05 (q, 2H, OCH_2CH_3), 3.44-3.24 (A part of AB spectrum, 1H, C(4)Ha), 2.71-1.90 (B part of AB spectrum, 1H, C(4)Hb and AB part of ABX spectrum, 2H, C(1)-CH_2), 2.33 (s, 3H, p-CH_3), 1.86 (br s, 1H, OH), 1.60 and 1.36 (2xs, 6H, 2xCH_3), 1.15 (t, 3H, OCH_2CH_3).

8-(2'-Hydroxy-2'-methylpropyl)-7,8,14,14a-tetrahydro-pyrrolidino-[1,2-c]-piperazino-[1',6'--2,3]-β-carboline-(5aHα, 8Hα, 14aHβ)-1,6-dione (22) and 8-(2'-Hydroxy-2'-methylpropyl)--7,8,14,14a-tetrahydro-pyrrolidino-[1,2-c]-piperazino-[1',6'-2,3]-β-carboline-(5aHα, 8Hβ, 14aHα)-1,6-dione (23)

Method a: A solution of **8a** (680 mg, 1.97 mmol) and Et_3N (200 mg, 1.98 mmol) in dry CH_2Cl_2 (10 mL) was added dropwise to a cooled (-20°C) stirring solution of L-TrOC-Pro-Cl[15] (2.43 mmol) in dry CH_2Cl_2 (20 mL) in an argon atmosphere. The reaction mixture was allowed to warm to room temperature and was monitored by TLC (solvent system A; **15** R_f 0.53.; **14** R_f 0.49). After one hour the reaction was completed, and the reaction mixture was washed succesively with 0.1 N aqueous HCl, 0.1 N $NaHCO_3$ and brine and dried with Na_2SO_4. Evaporation of the solvent *in vacuo* gave a

crystalline material, which was subjected to flash chromatography ($CHCl_3$/MeOH, 99.7/0.3, v/v) to yield 527 mg (43%) of **15** and 415 mg (34%) of **14**. Removal of the N-protecting group was accomplished quantitatively by refluxing the dipeptides in methanol (50 mL) with zinc dust for 10 minutes. Filtration and evaporation of the solvent gave 336 mg (43%) of **23** and 266 mg (34%) of **22**. Yields are based on **8a**.

Method b: Coupling of **8a** (840 mg, 2.43 mmol) with L-CBz-Pro-Cl[2] as described above and subsequently flash chromatography ($CHCl_3$/MeOH, 99.25/0.75, v/v) gave 610 mg (44%) of **28** (R_f 0.62, solvent system A) and 492 mg (35%) of **27** (R_f 0.53, solvent system A). Of these N-protected dipeptides the CBZ-group was removed by catalytic hydrogenation using Pd-C in ethanolic solution at atmospheric pressure. Filtration and evaporation of the solvent gave 403 mg (44%) of **23** and 320 mg (32%) of **22**.

Compound 22: R_f 0.26 (solvent system B); mp 270-271°C ($CHCl_3$/n-hexane); $[\alpha]_D^{22}$ +112 (c=1.25, methanol); UV (methanol) λmax 225, 267, 297 nm, λmin 251, 281 nm; EIMS (70 eV) m/z (relative intensity) 397 (M^+, 46%), 379 (8%), 324 (100%), 199 (42%); exact mass for $C_{22}H_{27}N_3O_4$ calcd. 397.2002, found 397.2003; ^{1}H NMR δ 8.69 (br s, 1H, NH), 7.33-6.68 (m, 2H, C(12)-C(13)H), 6.81 (s, 1H, C(10)H), 5.93 (t, 1H, ^{3}J=5.4 Hz, C(8)H), 4.36 (X part of ABX spectrum, 1H, C(14a)H), 4.24-3.97 (m, 1H, C(5a)H), 3.85-3.51 (m, 2H, C(3)H_2), 3.82 (s, 3H, OCH_3), 3.30 and 2.89 (AB part of ABX spectrum, 2H, ^{2}J=15.3 Hz, ^{3}J=3.9 Hz, ^{3}J=11.7 Hz, C(14)H_2), 2.60-1.68 (m, 7H, C(4)H_2-C(5)H_2, C(8)-C(1')H_2 and OH), 1.43 and 1.34 (2xs, 6H, 2xCH_3). Addition of the chiral shift reagent tris[3-((heptafluoropropyl)hydroxymethylene)-*d*-camphorato]europium (III) to the $CDCl_3$ solution of **22** did not cause splitting of signals. Anal. Calcd. for $C_{22}H_{27}N_3O_4$. 0.4$CHCl_3$ (Mw 445.229): C, 60.43; H, 6.20; N, 9.44. Found: C, 60.28; H, 6.17; N, 9.43.

Compound 23: R_f 0.26 (solvent system B); mp 234-236°C ($CHCl_3$/n-hexane); $[\alpha]_D^{22}$ -159 (c=2.0, methanol); UV (methanol) λmax 225, 267, 297 nm, λmin 250, 278 nm; E_HIMS (70 eV) m/z (relative intensity) 397 (M^+, 36%), 379 ($[C_{22}H_{25}N_3O_3]^{+\cdot}$ 5%), 324 ($C_{18}H_{18}N_3O_3]^+$, 100%), 199 (38%); exact mass for $C_{22}H_{27}N_3O_4$, calcd. 397.2002, found 397.1996; ^{1}H NMR δ 9.09 (br s, 1H, NH), 7.40-6.71 (m, 2H, C(12)-C(13)H), 6.82 (s, 1H, C(10)H, 6.00-5.87 (X part of ABX spectrum, 1H, C(8)H), 4.40 (X part of ABX spectrum, 1H, C(14a)H), 4.23-3.33 (m, 3H, C(5a)H and C(3)H_2), 3.83 (s, 3H, OCH_3), 2.95-1.73 (m, 9H, 2x AB part of ABX spectrum C(14)H_2 and C(8)-C(1')H_2, C(4)H_2-C(5)H_2 and OH), 1.53 and 1.35 (2xs, 6H, 2xCH_3). Addition of the chiral shift reagent tris[3-((heptafluoropropyl)hydroxymethylene)-*d*-camphorato]europium (III) to the $CDCl_3$ solution of **23** did not cause splitting of signals. Anal. Calcd. for $C_{22}H_{27}N_3O_4$. 0.8 $CHCl_3$ (Mw 492.981) : C, 55.55; H, 5.68; N, 8.52. Found: C, 55.28; H, 5.64; N, 8.48.

8-(2'-Hydroxy-2'-methylpropyl)-7,8,14,14a-tetrahydro-pyrrolidino-[1,2-c]-piperazino-[1',6'--2,3]-β-carboline-(5aHα, 8Hα, 14aHα)-1,6-dione (24) and 8-(2'-Hydroxy-2'-methylpropyl)--7,8,14,14a-tetrahydro-pyrrolidino-[1,2-c]-piperazino-[1',6'-2,3]-β-carboline-(5aHα, 8Hβ, 14aHβ)-1,6-dione (25)

Method a: Compound **9a** (155 mg, 0.45 mmol) was coupled with L-CBz-Pro-Cl (0.56 mmol) as described for the preparation of **22** abd **23**. This procedure gave 75 mg (29%) of **29** and 110 mg (42%) of **30**. The N-protecting group of these dipeptides was removed by catalytic hydrogenation using Pd-C in ethanolic solution at room temperature and atmospheric pressure to yield **20** and **21**, respectively. Cyclisation to the corresponding dioxopiperazines did not occur under these conditions. This was achieved by refluxing a solution of the amines **20** and **21** in ethanol to give 18 mg (10%) of **24** and 18 mg (10%) of **25**.

Method b (Epimerisation of N-protected dipeptides 14 and 15): A solution of **14** or **15** (325 mg, 0.53 mmol) and DBU (85 mg, 0.53 mmol) in dry $CHCl_3$ was stirred at 45°C in an argon atmosphere. Monitoring of the reaction by TLC showed that after 48 h. the equilibrium was reached. The reaction mixture was washed with 0.1 N aqueous HCl, brine and dried with Na_2SO_4. After evaporation of the solvent the residue was subjected to flash chromatography ($CHCl_3$/MeOH, 99.5/0.5, v/v) to yield 162 mg (50%) of **16** (R_f 0.42, solvent sytem B) and 162 mg starting material **14** (or 217 mg (67%) of **17** (R_f 0.40, solvent system B) and 108 mg starting material **15**). Removal of the N-protecting group and cyclisation to the diketopiperazines **24** and **25** was accomplished by refluxing a solution of **16** or **17** in a methanol (5 mL) with zinc dust for 3 days. After filtration and evaporation of the solvent, the residue was subjected to flash chromatography ($CHCl_3$/MeOH, 99.5/0.5, v/v) to yield 65 mg (63%) of **24** (or 63 mg (45%) of **25**).

Compound 24: Crystallisation attempts were unsuccesful. R_f 0.34 (solvent system B); $[\alpha]^{22}_D$ -62.5 (c=3.8, methanol); UV (methanol) λmax 226, 267, 297 nm, λmin 250, 280 nm; EIMS (70 eV) m/z

(relative intensity) 397 (M^+, 42%), 279 (6%), 324 (100%), 199 (38%); exact mass for $C_{22}H_{27}N_3O_4$ calcd. 397.2002, found 397.1998; ^1H NMR δ 8.81 (br s, 1H, NH), 7.43-6.67 (m, 2H, C(12)-C(13)H), 6.85 (s, 1H, C(10)H), 5.67 (X part of ABX spectrum, 1H, C(8)H), 4.20-4.00 (m, 2H, C(5a)H and X part of ABX spectrum C(14a)H), 3.80 (s, 3H, OCH_3), 3.72-2.93 (m, 4H, C(3)H_2 and AB part of ABX spectrum C(14)H_2), 2.49-1.64 (m, 7H, C(4)H_2-C(5)H_2, AB part of ABX spectrum C(8)-C(1')H_2 and OH), 1.38 and 1.13 (2xs, 6H, 2xCH_3).

Compound 25: Crystallisation attempts were unsuccesful. R_f 0.34 (solvent system B); $[\alpha]^{22}_D$ +30 (c=4.3, methanol); UV (methanol) λmax 225, 267, 297 nm, λmin 249, 281 nm; EIMS (70 eV) m/z (relative intensity) 397 (M^+, 40%), 479 (7%), 324 (100%), 199 (45%); exact mass for $C_{22}H_{27}N_3O_4$ calcd. 397.2002, found 397.2000; ^1H NMR δ 9.56 (br s, 1H, NH), 7.35-6.71 (m, 2H, C(12)-C(13)H), 6.81 (s, 1H, C(10)H), 4.94-4.73 (X part of ABX spectrum, 1H, C(8)H), 4.34-3.98 (m, 2H, C(5a)H and X part of ABX spectrum C(14a)H), 3.82 (s, 3H, OCH_3), 3.84-1.71 (m, 11H, C(3)H_2, AB part of ABX spectrum C(14)H_2, AB part of ABX spectrum C(8)-C(1')H_2, C(4)H_2-C(5)H_2 and OH), 1.45 and 1.41 (2xs, 6H, 2xCH_3).

Dehydration of 22-25

General method: To a stirred and cooled (-40°C) solution of the alcohol in dry pyridine (1 mL per 0.1 mmol of alcohol) was added freshly distilled thionyl chloride (1.5 equivalents) in an argon atmosphere. The reaction was monitored by TLC (solvent system C). The solution was allowed to warm to room temperature. After dilution with CH_2Cl_2 the resulting mixture was washed with 2 N aqueous HCl, brine and dried with Na_2SO_4. Evaporation of the solvent gave a mixture of reaction products, which were separated by flash chromatography.

Reaction of 22 (100 mg, 0.25 mmol) with $SOCl_2$ (44mg, 0.375 mmol) gave after flash chromatography (EtOAc/n-hexane, 60/40, v/v) 7 mg (7%) of **35**, 10 mg (11%) of **31** and 62 mg (65%) of **2a**.

8-(2'-methyl-1'-propenyl)-7,8,14,14a-tetrahydro-pyrrolidino-[1, 2-c]-piperazino-[1',6'-2,3]--β-carboline-(5aHα, 8Hα, 14aHβ)-1,6-dione (2a): R_f 0.42 (solvent system C); mp 261-263°C (EtOAc/n-hexane); $[\alpha]^{22}_D$ +250 (c=0.9, methanol); UV (methanol) λmax 230, 268, 297 nm, λmin 255, 282 nm; EIMS (70 eV) m/z (relative intensity) 379 (M^+, 82%), 324 (37%), 281 (100%), 199 (20%); ^1H NMR δ 7.76 (br s ,1H, NH), 7.32 (br d, 1H, C(13)H), 6.82 (s, 1H, C(10)H), 6.76 (m, 1H, C(12)H), 6.44 (d, 1H, ^3J=9.3 Hz, C(8)H), 5.34 (d, 1H, ^3J=9.3 Hz, C(1')H), 4.40 (X part of ABX spectrum, 1H, C(14a)H), 4.27-3.98 (m, 1H, C(5a)H), 3.82 (s, 3H, OCH_3), 3.82-3.50 (m, 2H, C(3)H_2), 3.27 and 2.87 (AB part of ABX spectrum, 2H, ^2J=15.0 Hz, ^3J=3.9 Hz, ^3J=11.7 Hz, C(14)H_2), 2.51-1.98)m, 4H, C(4)H_2-C(5)H_2), 1.96 and 1.75 (2xs, 6H, 2xCH_3); Anal. Calcd. for $C_{22}H_{25}N_3O_3$ (Mw 379.463): C, 69.64; H, 6.64; N, 11.07. Found: C, 69.49; H, 6.69; N, 10.92.

8-(2'-methyl-2'-propenyl)-7,8,14,14a-tetrahydro-pyrrolidino-[1, 2-c]-piperazino-[1',6'-2,3]--β-carboline-(5aHα, 8Hα, 14aHβ)-1,6-dione (31): R_f 0.46 (solvent system C); oil; EIMS (70 eV) m/z (relative intensity) 379 (M^+, 13%), 324 (100%), 199 (29%); ^1H NMR δ 7.86 (br s, 1H, NH), 7.39-6.74 (m, 2H, C(12)-C(13)H), 6.83 (s, 1H, C(10)H), 5.90 (t, 1H, ^3J=7.2 Hz, C(8)H), 4.93 (br d, 2H, ^2J=7.8 Hz, C(3')H_2), 4,42 (X part of ABX spectrum, 1H, C(14a)H), 4.20-4.03 (m, 1H, C(5a)H), 3.83 (s, 3H, OCH_3), 3.83-3.53 (m, 2H, C(3)H_2), 3.32 and 2.88 (AB part of ABX spectrum, 2H, ^2J=15.3 Hz, ^3J=3.3 Hz, ^3J=12.0 Hz, C(14)H_2), 2.54 (d, 2H, ^3J=7.2 Hz, C(1')H_2), 2.54-1.68 (m, 4H, C(4)-C(5)H_2), 1.91 (s, 3H, CH_3).

8-(2'-chloro-2'-methylpropyl)-7,8,14,14a-tetrahydro-pyrrolidino -[1,2-c]-piperazino-[1',6'-2,3]--β-carboline-(5aHα, 8Hα, 14aHβ)-1,6-dione (35): R_f 0.52 (solvent system C); oil, FABMS (7 kV at 1.4 mA) m/z (relative intensity) 418 ($[M+3]^+$, 2.5%), 416 ($[M+1]^+$, 6.6%), 185 (100%); ^1H NMR δ 8.80 (br s, 1H, NH), 7.37-6.74 (m, 2H, C(12)-C(13)H), 6.83 (s, 1H, C(10)H), 6.09 (t, 1H, ^3J=4.5 Hz, C(8)H), 4.40 (X part of ABX spectrum, 1H, C(14a)H), 4.23-4.02 (m, 1H, C(5a)H), 3.87-3.57 (m, 2H, C(3)H_2), 3.83 (s, 3H, OCH_3), 3.27 and 2.87 (AB part of ABX spectrum, 2H, ^2J=15.0 Hz, ^3J=3.8 Hz, ^3J=11.4 Hz, C(14)H_2), 2.60-1.60 (m, 4H, C(4)-C(5)H_2), 2.60 (d, 2H, ^3J=4.5 Hz, C(1')H_2), 1.72 and 1.68 (2xs, 6H, 2xCH_3).

Reaction of 23 (100 mg, 0.25 mmol) and $SOCl_2$ (44 mg, 0.375 mmol) gave after flash chromatography (EtOAc/n-hexane, 55/45, v/v) 12 mg (12%) of **36**, 12 mg (13%) of **32** and 27 mg (28%) of **3a**.

8-(2'-methyl-1'-propenyl)-7,8,14,14a-tetrahydro-pyrrolidino-[1, 2-c]-piperazino-[1',6'-2,3]--β-carboline-(5aHα, 8Hβ, 14aHα)-1,6-dione (3a): R_f 0.37 (solvent system C); mp 259-269°C

(EtOAc/n-hexane); $[\alpha]^{22}_D$ -327 (c=0.8, methanol); UV (methanol) λmax, 225, 270, 297 nm, λmin 254, 281 nm; EIMS (70 eV) m/z (relative intensity) 379 (M^+, 98%), 324 (100%), 199 (46%); ^{1}H NMR δ 7.68 (br s, 1H, NH), 7.36 (br d, 1H, C(13)H), 6.83 (s, 1H, C(10)H), 6.83-6.73 (m, 1H, C(12)H), 6.38 (d, 1H, ^{3}J=9.8 Hz, C(8)H), 5.23 (2, 1H, ^{3}J=9.8 Hz, C(1')H), 4.44 (X part of ABX spectrum, 1H, C(14a)H), 4.22-4.00 (m, 1H, C(5a)H), 3.95-3.33 (m, 2H, C(3)H_2), 3.82 (s, 3H, OCH_3), 3.09-1.90 (m, 6H C(4)-C(5)H_2 and AB part of ABX spectrum C(14)H_2), 2.03 and 1.76 (2xs, 6H, 2xCH_3); Anal. Calcd. for $C_{22}H_{25}N_3O_3$ (Mw 379.463): C, 69.64; H, 6.64; N, 11.07. Found: C, 69.33; H, 6.68; N, 10.88.

8-(2'-methyl-2'-propenyl)-7,8,14,14a-tetrahydro-pyrrolidino-[1, 2-c]-piperazino-[1',6'-2,3]--β-carboline-(5aHα, 8Hβ, 14aHα)-1,6-dione (32):R_f 0.42 (solvent system C); oil; EIMS (70 eV) m/z (relative intensity) 379 (M^+, 15%), 324 (100%), 199 (35%); ^{1}H NMR δ 7.96 (br s, 1H, NH), 7.42-6.75 (m, 2H, C(12)-C(13)H), 6.84 (s, 1H, C(10)H), 5.92 (t, 1H, ^{3}J=6.6 Hz, C(8)H), 4.91 (d, 2H, ^{2}J=7.5 Hz, C(3')H_2), 4.44 (X part of ABX spectrum, 1H, C(14a)H), 4.24-4.00 (m, 1H, C(5a)H), 3.94-3.33 (m, 2H, C(3)H_2), 3.84 (s, 3H, OCH_3), 3.04-1.62 (m, 6H, C(4)-C(5)H_2 and AB part of ABX spectrum C(14)H_2), 2.55 (d, 2H, ^{3}J=6.6 Hz, C(1')H_2), 1.89 (s, 3H, CH_3).

8-(2'-chloro-2'-methylpropyl)-7,8,14,14a-tetrahydro-pyrrolidino -[1,2-c]-piperazino-[1',6'-2,3]--β-carboline-(5aHα, 8Hβ, 14aHα)-1,6-dione (36): R_f 0.48 (solvent system C); oil; FABMS (7 kV at 1.4 mA) m/z (relative intensity) 418 ($[M+3]^+$, 2%), 416 ($[M+1]^+$, 5%), 185 (100%); ^{1}H NMR δ 8.23 (br s, 1H, NH), 7.37-6.74 (m, 2H, C(12)-C(13)H), 6.83 (s, 1H, C(10)H), 6.23 (t, 1H, ^{3}J=5.4 Hz, C(8)H), 4.49 (X part of ABX spectrum, 1H, C(14a)H), 4.28-4.00 (m, 1H, C(5a)H), 3.94-3.31 (m, 2H, C(3)H_2), 3.83 (s, 3H, OCH_3), 3.3-1.91 (m, 6H, C(4)-C(5)H_2 and AB part of ABX spectrum C(14)H_2), 2.31 (d, 2H, ^{3}J=5.4 Hz, C(1')H_2), 1.71 (s, 6H, 2xCH_3).

Reaction of 24 (50 mg, 0.12 mmol) and $SOCl_2$ (19 mg, 0.185 mmol) gave after flash chromatography (EtOAc/n-hexane, 50/50, v/v) 20 mg (44%) of **33** and 2 mg (4%) of **1a**.

Fumitremorgin C (1a). R_f 0.13 (solvent system D); oil; $[\alpha]^{22}_D$ -9 (methanol c=0.65); UV (methanol) λmax 225, 270, 296 nm ,λmin 255, 280 nm; EIMS (70 eV) m/z (relative intensity) 379 (M^+, 72%), 324 (100%), 199 (52%);^{1}H NMR (200 MHz) δ 7.66 (br s, 1H, NH), 7.44 (d, 1H, C(13)H), 6.86-6.79 (m, 2H, C(10)H and C(12)H), 5.98 (d, 1H, ^{3}J=9.6 Hz, C(8)H), 4.91 (dt, 1H, ^{3}J=9.6 Hz, ^{4}J=1.5 Hz, C(1')H), 4.23-4.08 (m, 2H, C(14a)H and C(5a)H), 3.84 (s, 3H, OCH_3), 3.68-3.60 (m, 1H, C(3)H_2), 3.51 and 3.10 (AB part of ABX spectrum, 2H, ^{3}J=6.3 Hz, ^{3}J=12.3 Hz, ^{2}J=18.0 Hz, C(14)H_2), 2.46-1.91 (m, 4H, C(4)-C(5)H_2), 2.00 (d, 3H, ^{4}J=1.5 Hz, CH_3), 1.66 (d, 3H, ^{4}J=1.5 Hz, CH_3).

8-(2'-methyl-2'-propenyl)-7,8,14,14a-tetrahydro-pyrrolidino-[1, 2-c]-piperazino-[1',6'-2,3]--β-carboline-(5aHα, 8Hα, 14aHα)-1,6-dione (33): R_f 0.14 (solvent system D);EIMS (70 eV) m/z (relative intensity) 379 (M^+, 14%), 324 (100%), 199 (42%); ^{1}H NMR δ 8.00 (br s, 1H, NH), 7.42 (d, 1H, C(13)H), 6.84 (s, 1H, C(10)H), 6.78 (d, 1H, C(12)H), 5.40 (X part of ABX spectrum, 1H, C(8)H), 4.64 (br d, 2H, ^{2}J=22.0 Hz, C(3')H_2), 4.20-3.97 (m, 2H, C(5a)H and C(14a)H), 3.84 (s, 3H, OCH_3), 3.67-1.73 (m, 10H, C(3)H_2, C(14)H_2, C(1')H_2 and C(4)-C(5)H_2), 1.64 (s, 3H, CH_3).

Reaction of 25 (40 mg, 0.1 mmol) and $SOCl_2$ (18 mg, 0.15 mmol) gave after flash chromatography (EtOAc/n-hexane, 60/40, v/v) 6 mg (16%) of **34** and 2 mg (5%) of **4a**.

8-(2'-methyl-1'-propenyl)-7,8,14,14a-tetrahydro-pyrrolidino-[1, 2-c]-piperazino-[1',6'-2,3]--β-carboline-(5aHα, 8Hβ, 14aHβ)-1,6-dione (4a): R_f 0.13 (solvent system D); EIMS (70 eV) m/z (relative intensity) 379 (M^+, 83%), 324 (100%), 199 (35%); ^{1}H NMR δ 7.85 (br s, 1H, NH), 7.42 (d, 1H, C(13)H), 6.85 (s, 1H, C(10)H), 6.73 (d, 1H, C(12)H), 5.54 (d, 1H, ^{3}J=9.0 Hz, C(8)H), 4.95 (d, 1H,^{3}J=9.0 Hz, C(1')H), 4.31-4.01 (m, 2H, C(14a)H and C(5a)H), 3.85 (s, 3H, OCH_3), 3.84-2.74 (m, 4H, C(3)H_2 and C(14)H_2), 2.45-1.75 (m, 4H, C(4)-C(5)H_2), 1.92 and 1.82 (2xs, 6H, 2xCH_3).

8-(2'-methyl-2'-propenyl)-7,8,14,14a-tetrahydro-pyrrolidino-[1, 2-c]-piperazino-[1',6'-2,3]--β-carboline-(5aHα, 8Hβ, 14aHβ)-1,6-dione (34): R_f 0.16 (solvent system D); EIMS (70 eV) m/z (relative intensity) 379 (M^+, 21%), 324 (100%), 199 (54%); ^{1}H NMR δ 8.26 (br s, 1H, NH), 7.37 (m, 1H, C(13)H), 6.83 (s, 1H, C(10)H), 6.77 (m, 1H, C(12)H), 5.10-4.85 (m, 1H, C(8)H), 4.96 (br d, 1H,^{2}J=8.1 Hz, C(3')H_2), 4.33-4.00 (m, 2H, C(14a)H and C(5a)H), 3.83 (s, 3H, OCH_3), 3.79-2.81 (m, 4H, C(3)H_2 and C(14)H_2), 2.53-1.67 (m, 6H, C(1')H_2 and C(4)-C(5)H_2), 1.80 (s, 3H, CH_3).

References and Notes

1. a) For the isolation report of fumitremorgin C -also called SM-Q-see: Cole, R.J.; Kirksey, J.W.; Dorner, J.W.; Wilson, D.M.; Johnson, J.C.; Johnson, A.N.; Bedell, D.M.; Springer, J.P.; Chexal, K.K.; Clardy, J.C.; Cox, R.H. *J. Agric. Food Chem.* **1977**, *25*, 826. b) Cole, R.J. In *Mycotoxins*

in Human and Animal Health: Pathotoxicology, Park Forest South, IL **1977**, p 583. c) Cole, R.J. *J. Food Protection* **1981**, *44*, 715. d) Yamazaki, M. In *Biosynthesis of Mycotoxins*; Steyn, P.S., Ed.; Academic: London, **1980**; p.204. e) Steyn,P.S.; Vleggaar, R.; Rabie, C.J. *Phytochemistry* **1981**, *20*, 538. f) Cole, R.J.; Cox, R.H. *Handbook of Toxic Fungal Metabolites*, Academic Press, London, **1981**, p. 355.

2. The stereochemistry at C(14a) of fumitremorgin C is not given in the only report available on the structure elucidation by single-crystal x-ray crystallography (see ref. 1b). Unfortunately, the crystallographic data cannot be traced anymore (Clardy, personal communication), and authentic material is hard to come by.
3. Plate, R.; Hermkens, P. H. H.; Smits, J. M. M.; Ottenheijm, H. C. J. *J. Org. Chem.* **1986**, *51*, 309.
4. Plate, R.; Hermkens P. H. H.; Behm, H.; Ottenheijm, H. C. J. *J. Org. Chem.* **1987**, *52*, 560.
5. Plate, R.; Hermkens, P.H.H.; Smits, J.M.M.; Nivard, R.J.F.; Ottenheijm, H.C.J. *J. Org. Chem.* **1987**, *52*, 1047.
6. Contrary to our earlier results, we were able to suppress the formation of β-carboline **12** by passing argon through the suspension.
7. Isomerisation of the compound **8b** under the same conditions was not observed.
8. Elimination and subsequent aromatisation of compounds related to **10** and **11** has been reported, see Harrison, D.M.; Sharma, R.B. *Tetrahedron Lett.* **1986**, *27* 521.
9. Elevated reaction temperatures led to considerable amounts of **12**.
10. In order to retain the homochirality of **13** during the coupling procedure, amine **8a** and triethylamine were added to a cooled (-20°C) solution of an excess of **13**.
11. Review: Hubert, A.J.; Reimlinger, H. *Synthesis* **1970**, 405.
12. Kodato, S-I.; Nakagawa, M.; Hongu, M.; Kawate, T.; Hino, T. *Tetrahedron*, **1988**, *44*, 359.
13. After having finished our synthesis of fumitremorgin C another total synthesis was published by; Hino, T.; Kawate, T.; Nakagawa, M. *Tetrahedron* **1989**, *45*, 1941.
14. Arx, E.v.; Faupel, M.; Bruggen, M. *J. Chromatogr.* **1976**, *120*, 224.
15. We prepared N-(2,2,2-trichloroethyloxycarbonyl)-L-proline acid chloride (**13**) as described (see ref. 4) for N-(benzyloxycarbonyl)-L-proline acid chloride (**26**). During the course of our studies a related reaction involving **13** was reported (see ref. 16).
16. Boyd, S.A.; Thompson, W.J. *J. Org. Chem.* **1987**, *52*, 1790.

Chapter 4.3

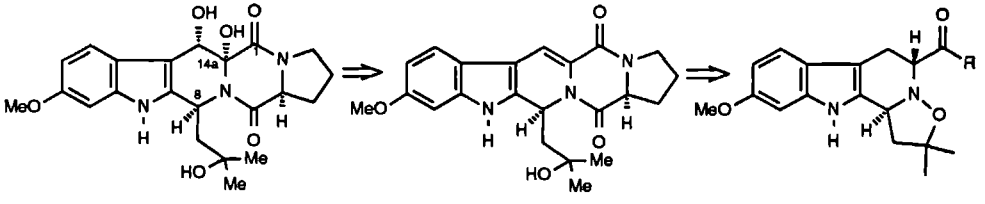
STEREOSELECTIVE SYNTHESIS OF (-)-VERRUCULOGEN TR-2.

In the previous chapter the total synthesis of the tremorgenic mycotoxin fumitremorgin C and three of its epimers has been presented.[1] The next target we settled upon was a more-functionalized member of the same class of natural products, *i.e.* verruculogen TR-2 (**1**).[2] So far a synthesis of this mycotoxin has not been reported.

Willingale *et.al.*[3] demonstrated by a labeling experiment that verruculogen TR-2 is biosynthetically derived from tryptophan, proline, methionine and mevalonate. Recently, it was suggested[4] that the biogenetic relationship between tryptophan on one hand and α-substituted- and α,β-dehydro-tryptophan derivatives on the other hand might proceed via N-hydroxytryptophan derivatives. Moreover, it was demonstrated[4] that N-hydroxytryptophan derivatives deserve attention as synthons in the preparation of natural products having α-functionalized- and α,β-dehydro-tryptophan as structural elements.

Based on these experiences we wondered whether the N-hydroxytryptophan derivative **3** could be converted into the α,β-dehydrotryptophan moiety present in **2**. This conversion might well be an avenue to the title compound, as the hydroxylation of α,β-dehydrotryptophan derivatives into the corresponding *cis*-diols has been reported before by others[5-8].

Scheme I

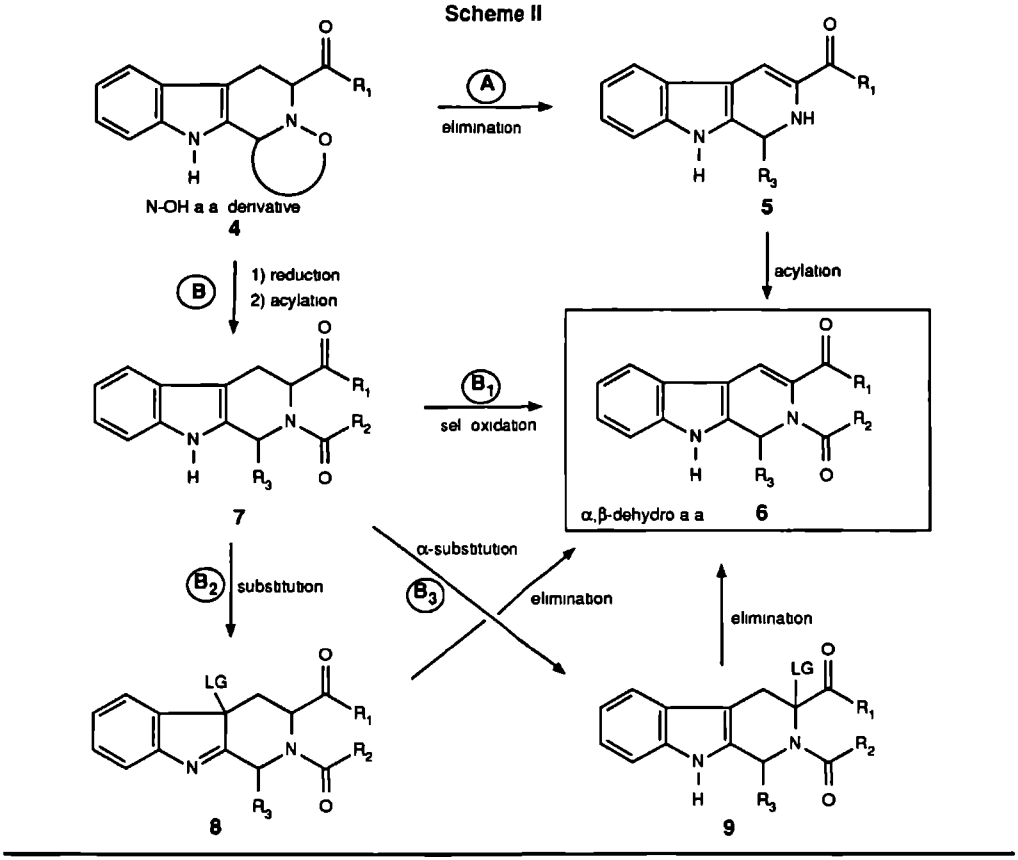


1 ***Verruculogen TR-2*** **2** **3**

Strategy

So the problem we faced here was the conversion of **3** into **2**. This is not a trivial reaction, as 1,2-dihydro-β-carbolines are relatively unstable and prone to undergo base-induced aromatization to give β-carbolines[9].

Starting from a tetrahydro-β-carboline we reasoned that two, fundamentally different approaches might yield a selective, formal conversion of **3** into **2** (Scheme II).



Initially, we studied the base-catalyzed elimination of the alkoxy group in N(2)-alkoxy derivatives (*e.g.* **4**) to give -after isomerization of the double bond- the dehydro β-carboline **5** (route A, Scheme II). Acylation of the amine function might then give the desired dehydro-β-carboline **6**.

Our second approach (route B) deviated from route A in that first the N-O bond was cleaved by reduction to yield a ring-opened amino alcohol of which the amino group was acylated to provide **7**. Subsequently several methods were studied for the formal dehydrogenation of the latter to give **6**.

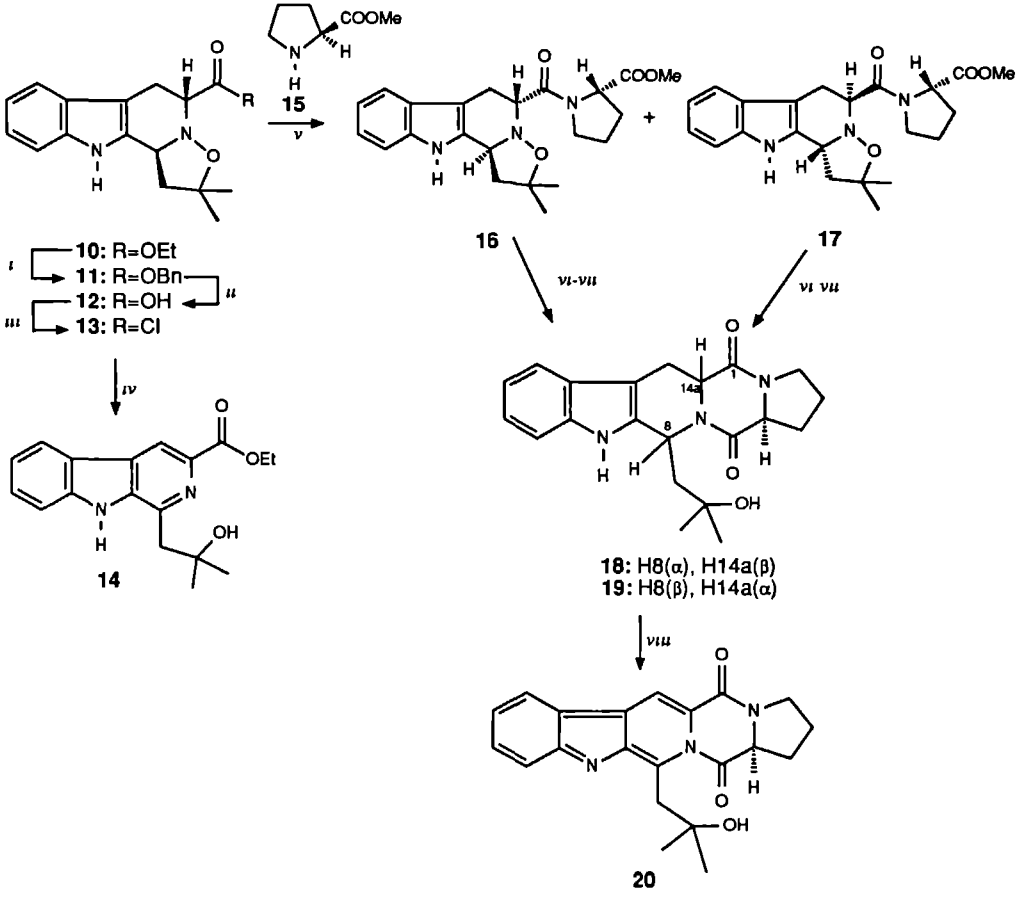
Firstly we studied a selective dehydrogenation of **7** with the oxidizing reagent DDQ (route B_1). A second method (route B_2) features the introduction of a leaving group at the 3-position of indole providing the indolenine **8**, which -after elimination of the leaving group and rearrangement- should give the dehydro compound **6** (route B_2). A third method (route B_3) has been explored successfully by Boyd[9], who introduced the benzeneseleninic function at the β-carboline C(3) position to yield **9** (LG=SeOPh) and subsequently eliminated this function to give **6**.

Initially, routes A, B_1 and B_2 were explored with a more readily available analogue of **3** lacking the methoxy substituent in the indole moiety. The most suitable approach to **2** was found to be route B_2, which was subsequently successfully applied in the total synthesis of **1**.

Results

Route A. In chapter 4.2 it is mentioned[10] that treatment of the isoxazolidine tetrahydro β-carboline **10** with base (NaH or KOtBu in dimethoxyethane) gave the aromatized β-carboline **14** instead of the desired dihydro compound (Scheme III). In a related reaction Harrison[11] has shown that this aromatization can be prevented by acylation of the NH function. We therefore set out to prepare the proline amides **16** and **17** anticipating that the proline ester moiety might capture intramolecularly the NH formed in the dihydro-β-carboline moiety to give the desmethoxy analogue of **2**. Firstly, the isoxazolidine fragment **10** had to be coupled with L-proline methyl ester. This coupling could be achieved by employing the acid chloride **13**, which was prepared from **10** in three steps. The first one was transesterfication of **10** by a mild method[12] using titanium (IV) isopropoxide in an excess of

Scheme III



i) $Ti(O_i C_3H_7)_4$ / BnOH ii) H_2, Pd/C iii) $(COCl)_2$, DMF / CH_2Cl_2 iv) NaH / DME v) Et_3N / CH_2Cl_2 vi) Zn / HOAc, 60°C vii) DBU / CH_2Cl_2 viii) DDQ / CH_2Cl_2

benzylalcohol to give the benzyl ester **11** (yield 92%). Chemoselective removal of the benzyl group was achieved by hydrogenation in the presence of catalytic Pd/C to give **12** quantitatively.[13] The carboxylic acid was converted into the acid chloride **13** by using oxalyl chloride in dichloromethane in the presence of a catalytic amount of DMF.

In order to retain the homo-chirality of **13** during the coupling procedure a solution of L-proline methyl ester **15** and triethylamine were added together and dropwise to a cooled (-20°C) solution of **13**. This procedure provided (76% yield) the amides **16** and **17** in a ratio of 1:1, which were easily separated by column chromatography. In order to assign the relative stereochemistry of **16** and **17** these compounds were converted into the known pentacycles **18** and **19**[14].

Unfortunately, conversion of **16** and **17** to the α,β-dehydro-compound **5** failed. Treatment with several bases (MeOH/NaOMe, KOtBu/MeOH, NaH/DME, DBU/THF, pyridine, KOtBu/CH_2Cl_2) or acids (H_2SO_4/MeOH, HCl(g)/dioxane, $TiCl_4/CH_2Cl_2$, TMSI/CH_3CN) gave only untractable reaction mixtures or starting material. This failure blocked the -in our opinion- most elegant approach for the conversion **3**→**2**.

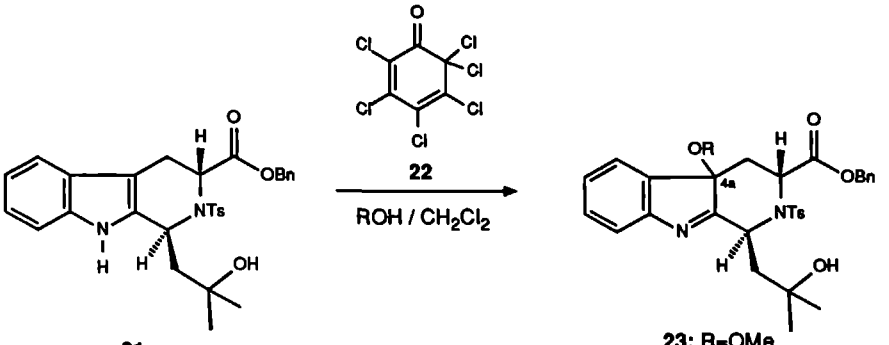
Route B_1. Attempted selective dehydrogenation of the pentacycles **18** and **19** with DDQ failed to give the corresponding 14,14a-dehydro derivative, *i.e.* the desmethoxy analogue of **2**. Reaction of **18** or **19** with DDQ (1 equiv.) in dichloromethane gave in stead 50% starting material and 50% of the N(2)-acyl-β-carboline anhydro base **20** (Scheme III). Subsequent addition of a second equivalent DDQ to the reaction mixture gave quantitatively **20**. The desired dehydro derivative **6** is probably an intermediate in this reaction. Attempts to prevent the over-oxidation (*i.e.* **6**→**20**) by temperature control or by the use of other solvents or by a milder oxidation reagent -such as *p*-chloranil- failed.

Route B_2. Electrophiles are easily incorporated at the 3-position of the indole unit of a tetrahydro-β-carboline[15]. Our initial investigation concerned the sulfonamide derivative **21**[1] as a model compound and 2,3,4,5,6,6-hexachlorocyclohexadien-1-one (**22**) as a mild Cl^+-donor[16]. The selectivity and mildness of this latter reagent is based on its ability to form a donor-acceptor complex and to form a hydrogen bond which leads to a well defined 'recognition' between this reagent and a substrate. It has however, not been studied so far in substitution reactions at an indole nucleus.

Suprisingly, we found that reaction of **21** in dichloromethane/methanol, 1/1, v/v with **22** at room temperature gave not the expected 3-chloroindolenine, but the 3-methoxyindolenine **23** in quantitative yield (Scheme IV). Reaction of **21** with **22** under identical conditions but now in a dichloromethane/ethanol or a dimethoxyethane/water mixture gave the 3-ethoxyindolenine **24** (80%) and the 3-hydroxyindolenine **25** (78%), respectively. Although examples of the introduction of a nucleophile at this position are extremely rare[17], we could not escape the conclusion that the above described conditions cause incorporation of a nucleophile at C(3) of the indole nucleus. Whereas this reaction proceeds with complete stereoselectivity we did not establish -for obvious reasons- the stereochemistry at C(4a) of the alkoxy indolenines **23-25**.

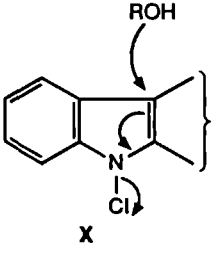
These findings can be rationalized as follows. Initially, **22** reacts with **21** to form a donor-acceptor

Scheme IV



complex, directed by a H-bridge between the indole NH proton and the oxygen of **22**. This is followed by the formation of pentachlorophenol and the N-chloro substituted intermediate **X**, which is now

Chart I

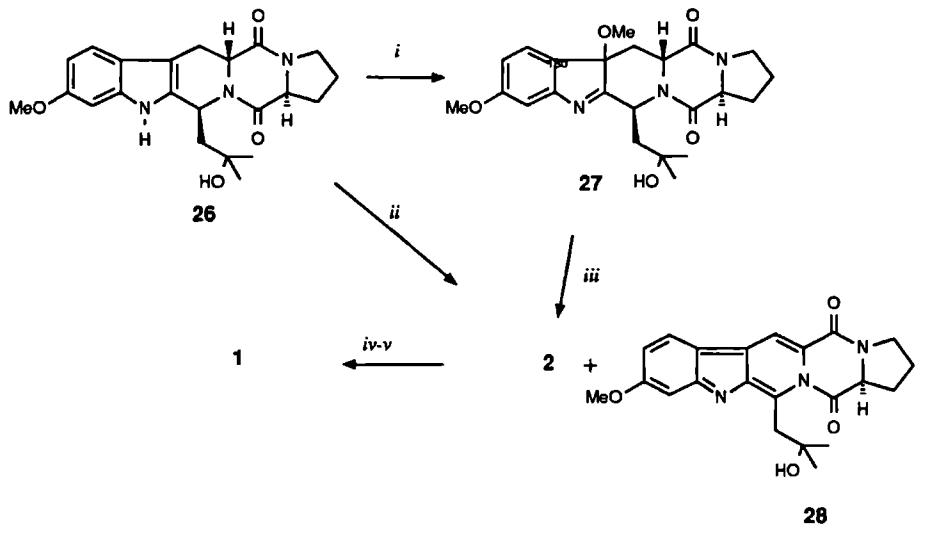


prone to undergo a nucleophilic attack at the indole C(3)-position (Chart I). The observed complete stereoselectivity renders a S_N1 type reaction less likely. It is also unlikely that first a 3-chloroindolenine is generated which subsequently undergoes nucleophilic substitution of the chlorine by ROH as neutral conditions are employed, whereas strongly alkaline[15d] or acidic[15f] conditions are reported to be required for this conversion.

Application of this methodology to the pentacycle **26**[1] -a precursor of one of the epimers of fumitremorgin C (see chapter 4.2)- gave the 3-methoxyindolenine **27** (73%) with complete stereoselectivity (Scheme V). Subsequently, a solution of **27** in dichloromethane and a few drops of trifluoroacetic acid was stirred at room temperature to give the dehydrodipeptide **2** (46%), together with the aromatized product **28** (6%) and recovered starting material **27** (43%). This mixture was separated easily by column chromatography whereupon the starting material **27** was treated again with TFA. This led to 80% overall yield of **2**.

Treatment of **26** with **22** in ethanol (neat) at room temperature gave directly the dehydrogenated product **2** in 37% yield. However, formation of the side-product **28** in up to 23% yield made this route less attractive.

Scheme V



i) CH_2Cl_2 / MeOH / 22 ii) EtOH / 22 iii) CH_2Cl_2 / TFA iv) OsO_4 / pyridine v) $NaHSO_3$

Next our strategy employed the transformation of the C(14)-C(14a) alkene moiety of key intermediate **2** into the *cis*-diol of the natural product **1**. Thus, based on literature procedures[8] using OsO_4 in pyridine, followed by a reductive work up with sodium bisulfite, **2** was converted into **1** in 22% yield (Scheme V). The spectroscopical data of **1** are identical to those of the natural verruculogen TR-2[2e].

Recently, the synthesis of desmethoxy TR-2 has been reported[8]. However, we are puzzled about the ^1H NMR spectrum of the compound reported in that study. It differs substantially from the spectrum we observed for **1**. Moreover, the sign of the specific rotation we measured for **1** ($[\alpha]_D$-45°) is opposite to that reported by Boyd and Thompson ($[\alpha]_D$+116°). Therefore we are tempted to conclude that these authors described the synthesis of one of the isomers of demethoxy TR-2.

Conclusion

Our approach to **1** constitutes the first total synthesis of verruculogen TR-2 and provides definite proof of its structure. Reaction of **26** with **22** in dichloromethane/methanol provides the methoxyindolenine **27**, which in the presence of TFA rearranges to the dehydrodipeptide **2**. This compound was elaborated to **1** employing osmium tetroxide followed by reductive work up.

Experimental Section

Melting points were taken on Koefler hot stage (Leitz-Wetzlar) and are uncorrected. Ultraviolet spectra were measured with a Perkon Elmer spectrophotometer, Model lambda 5. Proton magnetic resonance spectra were measured on a Bruker WH-90 or on a Bruker AM 200 spectrometer. Chemical shifts are reported as δ values (parts per million) relative to tetramethylsilane as an internal standard. Mass spectra were obtained with a double focusing VG 7070E spectrometer. Thin layer

chromatography (TLC) was carried out by using Merck precoated silicagel F-254 plates (thickness, 0.25 mm). Spots were visualized with a UV hand lamp, iodine vapor, Cl_2-TDM.[18] For column chromatography Merk silicagel 60H was used.

2,2-Dimethyl-5-(benzoxycarbonyl)-4,5,6,11b-tetrahydro-isoxazolidino[2,3-a]-β-carboline (11)
A solution of **10**[10] (3.7 g, 11.8 mmol), benzylalcohol (20 mL) and 0.3 equivalent titanium(IV)isopropoxide (Aldrich Chemical Co. 1.0 g, 3.5 mmol) in dioxane (30 mL) was kept for 18h at 100°C and argon atmosphere. The reaction was monitored by TLC ($CHCl_3$/MeOH, 99/1, v/v). After dilution with CH_2Cl_2 (100 mL) the mixture was washed with 1 N HCl. The troubled organic layer was filtered and the filtrate was washed with brine and dried with Na_2SO_4 and evaporated. Crystallization from $CHCl_3$/n-hexane gave 2.5 g (57%) of **11**. Removing of the benzylalcohol by vacuum destillation and crystallization of the resulting residue gave an extra 1.15 g (26%) product. Total yield 83%. ; mp 210-212°C ($CHCl_3$); R_f 0.64 ($CHCl_3$/MeOH, 93/7, v/v); CIMS (100 eV) m/z (relative intensity) 377 ($[M+1]^+$, 100%), 241 ($[C_{15}H_{17}N_2O]^+$, 91%), 91 ($[C_7H_7]^+$, 72%); exact mass for $C_{23}H_{25}N_2O_3$ calcd. 377.187, found 377.189; ^1H NMR ($CDCl_3$) δ 7.95 (br s, 1H, NH), 7.60-7.18 (m, 9H, C(7)-C(10)H and Ph), 5.45 and 5.30 (AB spectrum, 2H, ^2J=14 Hz, OCH_2), 5.03 (X part of ABX spectrum, 1H, C(11b)H), 4.28 (t, 1H, J=6.6 Hz, C(5)H), 3.21 (d, 2H, J=6.6 Hz, C(6)H_2), 2.60 and 2.38 (AB part of ABX spectrum, 2H, ^2J=21.3 HZ, J=6.3 Hz, J=9.9 Hz, C(1)H_2), 1.53 and 1.50 (2xs, 6H, 2xCH_3); Anal.Calcd. for $C_{23}H_{24}N_2O_3$: C, 73.38; H, 6.43; N, 7.44. Found: C, 73.03; H, 6.41; N, 7.32.

Compound 16 and Compound 17
Hydrogenation of **11** (1.28g, 3.4 mmol) in EtOH/CH_2Cl_2, 95/5, v/v (200 mL) using catalytic Pd-C at room temperature and atmospheric pressure gave 996 mg (100%) **12** as a crystalline material. As a solid and kept under argon it was stable. Rf=0.12 ($CHCl_3$/MeOH, 93/7, v/v); CIMS (100 eV) m/z 287 ($[M+1]^+$, 2%), 285 ($[M-1]^+$, 4%), 269 ($[M-OH]^+$, 3%), 241 ($[M-COOH]^+$, 20%), 183($[C_{12}H_{11}N_2]^+$, 24%), 169($[C_{11}H_9N_2]^+$, 19%), 59(100%); ^1H NMR (DMSO-d^6) δ 10.97 (br s, 1H, NH), 7.50-6.88 (m, 4H, C(7)-C(10)H), 4.79 (X part of ABX spectrum, 1H, C(11b)H), 4.30 (X part of ABX spectrum, 1H, C(5)H), 3.19-2.78 (AB part of ABX spectrum, 2H, C(6)H_2), 2.67-1.94(AB aprt of ABX spectrum, 2H, C(1)H_2), 1.34 (s, 6H, 2xCH_3). To a cooled (-20°C) and stirring mixture of **12** in dry CH_2Cl_2 (100 mL) and a few drops of DMF was added dropwise oxalylchloride (475 mg, 3.75 mmol). The reaction mixture became clear while CO and CO_2-evolution occurred. After completion of the reaction a solution of (L)-Pro-OMe (485 mg, 3.75 mmol) and Et_3N (760 mg, 7.52 mmol) in dry CH_2Cl_2 was added dropwise to the cooled stirring solution in an argon atmosphere. The reaction mixture was allowed to warm to room temperature. After completion of the reaction (1 h.) as was monitored by TLC ($CHCl_3$/MeOH, 93/7, v/v) the reaction mixture was successively washed with 0.1 N $NaHCO_3$, 0.1 N HCl and brine. The organic layer was dried (Na_2SO_4) and the solvent was evaporated in vacuo. The residue was subjected to column chromatography ($CHCl_3$/MeOH, 98.5/1.5, v/v) to give 530 mg (39%) of **16** and 498 mg (37%) of **17**.
Compound 16: Crystallized from MeOH/$CHCl_3$: mp 263-265°C; Rf 0.46 ($CHCl_3$/MeOH, 93/7, v/v); $[\alpha]^{22}_D$+4[2] (c=2.6, methanol); EIMS (70 eV) m/z (relative intensity) 397 (M^+, 2), 382 ($[M-CH_3]^+$, 8), 338 ($[M-COOMe]^+$, 6), 241 ($[C_{15}H_{17}N_2O]^+$, 100), 183 ($[C_{12}H_{11}N_2]^+$, 82); ^1H NMR δ 8.16 (br s, 1H, NH), 7.48-7.00 (m, 4H, C(7)-C(10)H), 4.81 (t, 1H, C(11b)H), 4.57 (t, 1H, C(5)H), 4.32 (t, 1H, C(2')HCOOMe), 3.94 (br t, 2H, C(5')H_2), 3.71 (s, 3H, OCH_3), 3.08 (d, 2H, C(6)H_2), 2.60-1.82 (m, 6H, C(1)H_2 and C(3')H_2-C(4')H_2), 1.36 (s, 6H, 2xCH_3); Anal. Calcd. for $C_{22}H_{27}N_3O_4$ (Mw 397.496): C, 66.48; H, 6.85; N, 10.57. Found: C, 66.80; H, 6.72; N, 10.30.
Compound 17: Crystallized from CH_2Cl_2/n-hexane: mp 286-288°C; Rf 0.43 ($CHCl_3$/MeOH, 93/7, v/v); $[\alpha]^{22}_D$-86[1] (c=1.6, methanol); EIMS (70 eV) m/z (relative intensity) 397 (M^+, 2), 241 ($[C_{15}H_{17}N_2O]^+$, 75), 183 ($[C_{12}H_{11}N_2]^+$, 100); ^1H NMR δ 7.87 (br s, 1H, NH), 7.54-7.07 (m, 4H, C(7)-C(10)H), 4.96 (t, 1H, C(11b)H), 4.67 (t, 1H, C(5)H), 4.37 (t, 1H, C(2')HCOOMe), 3.89-3.58 (m, 2H, C(5')H_2), 3.64 (s, 3H, OCH_3), 3.11 (d, 2H, C(6)H_2), 2.67-1.92 (m, 6H, C(1)H_2 and C(3')H_2-C(4')H_2), 1.39 and 1.32 (2xs, 6H, 2xCH_3); Anal. Calcd. for $C_{22}H_{27}N_3O_4$ (Mw 397.496): C, 66.48; H, 6.85; N, 10.57. Found: C, 66.12; H, 6.75; N, 10.41.

pentacyclic skeleton 18 and 19
To a warmed (50°C) stirring solution of **16** (or **17**) (40 mg, 0.1 mmol) in acetic acid was added activated zink dust. After completion of the reaction (30 minutes) as was monitored by TLC ($CHCl_3$/MeOH, 93/7, v/v) the reaction mixture was filtered and the filtrate was evaporated in vacuo. The residue was dissolved in dichloromethane and successively washed with 0.1N $NaHCO_3$ and brine.

The organic layer was dried (Na_2SO_4) and concentrated to dryness. The residue was disolved in dry dichloromethane and DBU (1 equiv.) was added. After completion of the reaction (3 days) as was monitored by TLC ($CHCl_3$/MeOH, 93/7, v/v) the reaction mixture was washed with 1N HCl and brine. The organic layer was dried (Na_2SO_4) and evaporation of the solvent in vacuo gave 35 mg (95%) of crystalline **18** (or **19**). Spectroscopical data are identical with earlier published results[14].

Oxidation attempt of 18 (or 19) with DDQ.

Compound 20

To a stirred solution of **18** (or **19**) (92 mg, 0.25 mmol) in dry dichloromethane (5 mL) was added dropwise DDQ (125 mg, 0.55 mmol) in dichloromethane (10 mL). After completion of the reaction (1 h.) as was monitored by TLC ($CHCl_3$/MeOH, 93/7, v/v) the reaction mixture was washed with 0.1N NaOH and brine. The organic layer was dried (Na_2SO_4) and concentrated to dryness. The resisue was subjected to column chromatography ($CHCl_3$/MeOH, 99/1, v/v) to give 86 mg (95%) of **20**; Rf 0.56 ($CHCl_3$/MeOH, 93/7, v/v); UV (MeOH) λmax 210, 237, 271, 285(sh), 304(sh), 316(sh), 335, 350 nm; EIMS (70 eV) m/z (relative intensity) 363 (M^+, 15), 222 (100); ^{1}H NMR δ 8.37 (s, 1H, C(14)H), 7.92 (d, 1H, C(10)H), 7.47 (m, 1H, C(13)H), 7.31-7.05 (m, 2H, C(11)-C(12)H), 4.11 (m, 1H, C(5a)H), 3.90-3.60 (m, 2H, C(3)H_2), 3.44 and 3.28 (AB spectrum, 2H, ^{2}J=16.0 Hz, C(15)H_2), 2.22-1.76 (m, 4H, C(4)H_2-C(5)H_2), 1.60 and 1.13 (2xs, 6H, 2xCH_3).

General procedure to 3-alkoxy-indolenines

A solution of 2,3,4,5,6,6-hexachloro-2,4-cyclohexandien-1-one (**22**; Janssen Chimica; 33 mg, 0.11 mmol) in dichloromethane (2 mL) was added dropwise to a stirred solution of **21**[1] (53 mg, 0.1 mmol) in CH_2Cl_2/ROH, 1/1, v/v (8 mL). After completion of the reaction (2-4 h.) as was monitored by TLC (EtOAc/n-hexane, 40/60, v/v) the reaction mixture was washed with 1N NaOH and brine. The organic layer was dried (Na_2SO_4) and concentrated to dryness. The residue was subjected to column chromatography ($CHCl_3$) to give the 3-alkoxy indolenines **23-25**.

Compound 23 (R=Me): Yield 56 mg (100%); oil; Rf 0.48 (EtOAc/n-hexane, 40/60, v/v); UV (MeOH) λmax 205, 231 nm; EIMS (70 eV) m/z (relative intensity) 562 (M^+, 14), 407 ([M-$SO_2C_7H_7$]$^+$, 34), 91 ([C_7H_7]$^+$, 100); ^{1}H NMR δ 7.78 (d, 2H, p-C_6**H**a_2Hb_2(CH_3)), 7.41-6.93 (m, 9H, C_6H_5, p-C_6Ha_2**H**b_2(CH_3) and C(6)-C(7)H), 6.78-6.40 (m, 2H, C(5)H and C(8)H), 4.74 (X part of ABX spectrum, 1H, C(1)H), 4.56 and 4.27 (AB spectrum, 2H, ^{2}J=12.4 Hz, OCH_2Ph), 4.21 (br t, 1H, C(3)H), 3.11 (s, 3H, OCH_3), 2.70-1.75 (m, 4H, C(4)H_2 and C(1)CH_2), 2.42 (s, 3H, p-C_6H_4(CH_3)), 1.58 (br s, 1H, OH (exchangeable)), 1.34 and 1.28 (2xs, 6H, 2xCH_3)

Compound 24 (R=Et): Yield 46 mg (80%); oil; Rf 0.58 (EtOAc / n-hexane, 40/60, v/v); UV (MeOH) λmax 207, 231 nm; EIMS (70 eV) m/z (relative intensity) 576 (M^+, 4), 421 ([M-$SO_2C_7H_7$]$^+$, 11), 91 ([C_7H_7]$^+$, 100); ^{1}H NMR δ 7.73 (d, 2H, p-C_6**H**a_2Hb_2(CH_3)), 7.32-6.90 (m, 9H, C_6H_5, p-C_6Ha_2**H**b_2(CH_3) and C(6)-C(7)H), 6.73-6.38 (m, 2H, C(5)H and C(8)H), 4.69 (X part of ABX spectrum, 1H, J=3.3Hz, J=5.9Hz, C(1)H), 4.50 and 4.34 (AB spectrum, 2H, ^{2}J=12.3 Hz, OCH_2Ph), 4.18 (X part of ABX spectrum, 1H, J=6.9Hz, J=11.7Hz, C(3)H), 3.30 (m, 2H, OCH_2CH_3), 2.76-1.71 (m, 4H, C(4)H_2 and C(1)CH_2), 2.38 (s, 3H, p-C_6H_4(CH_3)), 1.60 (br s, 1H, OH (exchangeable)), 1.33 and 1.27 (2xs, 6H, 2xCH_3)

Compound 25 (R=H): Instead of dichloromethane, dimethoxyethane was used as the solvent in this experiment. Yield 43 mg (78%); oil; Rf 0.41 (EtOAc/n-hexane, 40/60, v/v); UV (MeOH) λmax 205, 231 nm; EIMS (70 eV) m/z (relative intensity) 548 (M^+, 69), 413 ([M-$COOC_7H_7$]$^+$, 4), 393 ([M-$SO_2C_7H_7$]$^+$, 37), 337 (25), 230 (30), 91 ([C_7H_7]$^+$, 100); ^{1}H NMR δ 7.7$_7$ (d, 2H, p-C_6**H**a_2Hb_2(CH_3)), 7.36-6.97 (m, 9H, C_6H_5, -C_6Ha_2**H**b_2(CH_3) and C(6)-C(7)H), 6.81-6.44 (m, 2H, C(5)H and C(8)H), 4.93 and 4.76 (AB spectrum, 2H, ^{2}J=12.9 Hz, OCH_2Ph), 4.67-4.51 (2x X part of ABX spectrum, 2H, C(1)H and C(3)H), 4.12 (br s, 1H, C(4a)OH (exchangeable)), 2.56-1.88 (m, 4H, C(4)H_2 and C(1)CH_2), 2.39 (s, 3H, p-C_6H_4(CH_3)), 1.61 (br s, 1H, OH (exchangeable)), 1.34 (s, 6H, 2xCH_3).

Compound 26

A solution of 2,3,4,5,6,6-hexachloro-2,4-cyclohexandien-1-one (**22**: Janssen Chimica; 38 mg, 0.13 mmol) in dichloromethane (2 mL) was added dropwise to a stirred solution of **26**[1] (46 mg, 0.12 mmol) in CH_2Cl_2/MeOH, 1/1, v/v (15 mL). After completion of the reaction (30 min) as was monitored by TLC (EtOAc) the reaction mixture was washed with 1N NaOH and brine. The organic layer was dried (Na_2SO_4) and concentrated to dryness. The residue was subjected to column chromatography ($CHCl_3$) to give 37 mg (73%) of **27**. Colourless oil; Rf 0.28 (EtOAc); $[\alpha]_D$ +95 ° (c=3.5, methanol); UV

(methanol) λmax 210, 240, 296 nm; EIMS (70 eV) m/z (relative intensity) 427 (M^+, 35), 412 (22), 396 (4), 395 (3), 260 (37), 28 (100); ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.00 (d, 1H, C(13)H), 6.36-6.17 (m, 2H, C(10) and C(12)H), 5.45 (dd, 1H, C(8)H), 4.40 (dd, 1H, C(14a)H), 4.10 (br s, 1H, OH), 4.04-3.87 (m, 1H, C(5a)H), 3.69 (s, 3H, OCH_3), 3.53 (s, 3H, OCH_3), 3.78-3.29 (m, 2H, C(3)H_2), 2.60-1.16 (m, 8H, C(14)H_2, C(1')H_2, C(4)-C(5)H_2), 1.36 (s, 6H, 2xCH_3).

Dehydrodipeptide 2 and anhydro base 28

To a stirred solution of **27** (35mg, 0.082 mmol) in dichloromethane (20 mL) at room temperature was added a few drops of trifluoroacetic acid. Stirring was continued for 1h after which the reaction mixture was washed with 0.1N $NaHCO_3$ and brine, The organic layer was dried (Na_2SO_4) and evaporated to dryness. The residue was subjected to column chromatography (EtOAc) to give 15 mg (46%) of **2**, 15 mg (43%) of starting material **27** and 2 mg (6%) of **28**.

Compound 2: Amorphous yellow-green solid; R_f 0.14 (EtOAc); $[\alpha]_D$ +87° (c=1.3, methanol); UV (methanol) λmax 227, 254, 363 nm; EIMS (70 eV) 395 (M^+, 13), 322 (40), 294 (18), 225 (21), 197 (27), 57 (100); ^{1}H NMR (90 MHz, $CDCl_3$) δ 9.25 (br s, 1H, NH), 7.51 (d, 1H, C(13)H), 7.34 (s, 1H, C(14)H), 6.87 (s, 1H, C(10)H), 6.82 (m, 1H, C(12)H), 6,17 (t, 1H, C(8)H), 4.22-4.00 (m, 1H, C(5a)H), 3.89-3.54 (m, 2H, C(3)H_2), 3.82 (s, 3H, OCH_3), 2.53-1.71 (m, 6H, C(1')H_2, C(4)-C(5)H_2), 2.00 (br s, 1H, OH), 1.42 and 1.18 (2xs, 6H, 2xCH_3)

Compound 28: Rf 0.43 (EtOAc); UV (MeOH) λmax 217, 236, 272, 285(sh), 304(sh), 317(sh), 335, 354 nm; EIMS (70 eV) m/z (relative intensity) 393 (M^+, 18), 252 (100), 222 (86); ^{1}H NMR δ 8.38 (s, 1H, C(14)H), 7.93 (d, 1H, C(13)H), 6.96 (s, 1H, C(10)H), 6.90 (d, 1H, C(12)H), 4.13 (m, 1H, C(5a)H), 3.98-3.71 (m, 2H, C(3)H_2), 3.86 (s, 3H, OCH_3), 3.57 and 3.18 (AB spectrum, 2H, ^{2}J=14.5 Hz, C(15)H_2), 2.23-1.75 (m, 4H, C(4)H_2-C(5)H_2), 1.61 and 1.24 (2xs, 6H, 2xCH_3).

(-)-Verrucculogen TR-2 (1)

To a stirred and cooled (0°C) solution of the dehydro compound **2** (5 mg, 0.0127 mmol) in dry pyridine (0.5 mL) was added a solution of osmium tetroxide (100 µl, 0.0195 M in pyridine). The orange solution was stirred at 0°C for 2 h and the was treated with saturated aqueous $NaHSO_3$ (0.5 mL), and the mixture was allowed to react at room temperature for 30 min, at which time the aqueous layer separated. The mixture was extracted with chloroform and subsequently the organic layer washed with brine. The organic layer was dried (Na_2SO_4) and concentrated to dryness. The residue was subjected to column chromatography ($CHCl_3$/MeOH, 97/3, v/v) to give 1.2 mg (22%) of **1**. oil; R_f 0.39 ($CHCl_3$/MeOH, 93/7, v/v); $[\alpha]_D$ -45° (c=0.55, CH_2Cl_2); EIMS (70 eV) 429, (M^+, 4), 411 (8), 335 (45), 278 (47), 219 (100); UV (methanol) λmax 224, 267, 295 nm; ^{1}H NMR (200 MHz, $CDCl_3$) δ 9.04 (br s, 1H, NH), 7.82 (d, 1H, C(13)H), 6.86-6.77 (m, 2H, C(10)H and C(12)H), 5.72 (d, 1H, J=2.9 Hz, C(14)H), 5.46 (dd, 1H, J_{AX}+J_{BX}=11.2 Hz, C(8)H), 4.60 (d, 1H, J=2.9 Hz, C(14)OH, 4.45 (m, 1H, C(5a)H), 4.02 (d, 1H, J=2 Hz, C(14a)OH), 3.95 (s, 3H, OCH_3), 3.70-3.61 (m, 2H, C(3)H_2), 2.51 and 2.12-1.65 (m, 7H, C(1')H_2, C(4)-C(5)H_2), 1.55 (s, 3H, CH_3), 1.18 (s, 3H, CH_3); (90 MHz, DMSO-d_6) δ 10.55 (br s, 1H, NH), 7.60 (d, 1H, J=9 Hz, C(13)H), 6.83 (s, 1H, C(10)H), 6.58 (d, 1H, J=9 Hz, C(12)H), 6.13 (br s, 1H, OH), 5.48 (d, 1H, C(14)H), 5.32 (m, 1H, C(8)H, 5.16 (d, 1H, C(14)OH), 4.33 (m, 1H, C(5a)H), 4.17 (br s, 1H, OH), 3.70 (s, 3H, OCH_3), 3.55 (m, 2H, C(3)H_2), 2.36-1.60 (m, 6H, C(1')H_2, C(4)-C(5)H_2), 1.09 and 0.94 (2xs, 6H, 2x CH_3)

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14. Reductive ring opening of the isoxazolidine moiety of **16** and **17** by zinc dust in acetic acid at 50°C gave -after work-up and subsequent addition of the base DBU in chloroform- cyclization to the pentacycles **18** and **19** in respectively 87% and 83% yield. Comparison of the ^{1}H NMR data of **18** and **19** with those published earlier made the structure assignment possible: Plate, R.; Hermkens, P.H.H.; Behm, H.; Ottenheijm, H.C.J. *J. Org. Chem.* **1987**, *52*, 560.
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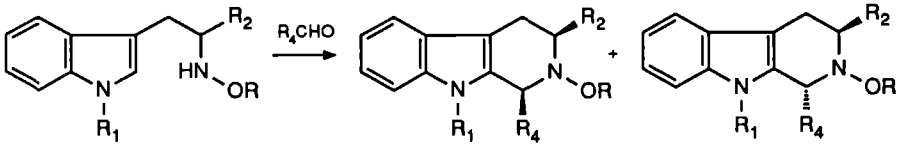
CHAPTER 5

Syntheses of 1,3-disubstituted N-hydroxy(alkoxy)-β-carbolines by the Pictet-Spengler reactions of N-hydroxy(alkoxy)-tryptophan and -tryptamine derivatives.



cis *trans*

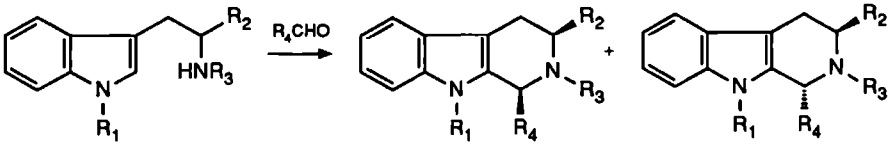
"Syntheses of 1,3-disubstituted N-oxy-β-carbolines by the Pictet-Spengler reactions of N-oxy-tryptophan and -tryptamine derivatives.", Pedro H.H. Hermkens, Jan H. v. Maarseveen, Peter L.H.M. Cobben, Harrie C.J. Ottenheijm, Chris G. Kruse, Hans W. Scheeren, *Tetrahedron* (**1990**), *45*, 833.

Chapter 5

SYNTHESES OF 1,3-DISUBSTITUTED N-HYDROXY(ALKOXY)-β-CARBOLINES BY THE PICTET-SPENGLER REACTIONS OF N-HYDROXY(ALKOXY)-TRYPTOPHAN AND -TRYPTAMINE DERIVATIVES.

The tetrahydro-β-carboline nucleus is a structural feature present in many indole alkaloids, and the Pictet-Spengler reaction (Scheme I) is the most widely used method for synthesizing this tricyclic system. Recent examples are the total synthesis of Pyridindolol[1], Fumitremorgin-Verruculogen[2] and Eudistomins[3]. For obvious reasons much attention has been focussed on stereochemical[1b,2,3b,4,5] and mechanistic[4b,6] aspects of this reaction.

Scheme I



1 R_1=H, R_2=COOMe, R_3=H
2. R_1=H, R_2=COOMe, R_3=Bn
3: R_1=H, R_2=COOEt, R_3=OH
4 R_1=H, R_2=COOEt, R_3=OBn
5: R_1=H, R_2=H, R_3=OH

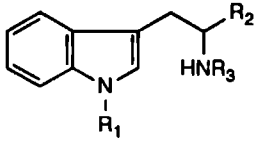
6 *(cis)*

7 *(trans)*

Generally, tryptophan methyl ester (**1**) and aldehydes in aprotic solvents yield both *cis* and *trans* 1,3-disubstituted-1,2,3,4-tetrahydro-β-carbolines **6** and **7** (Scheme I). It has been demonstrated[4g] that high *cis*-selectivity can be achieved if the Pictet-Spengler reaction is conducted at low temperatures (0°C). High or complete *trans*-selectivity was observed when N-benzyl tryptophan methyl ester **2** was condensed with aldehydes.[4b,4h]

In contrast, Pictet-Spengler reactions of derivatives of N-hydroxytryptophans[3b] (*i.e.* **3** and **4**) and

Chart I



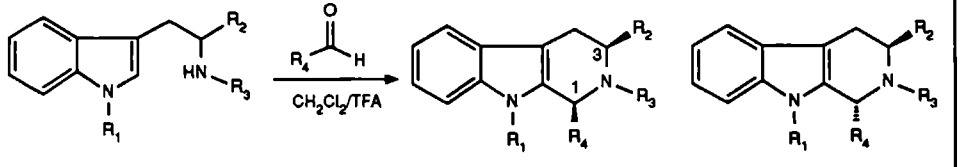
3: R_1=H, R_2=COOEt, R_3=OH
4 R_1=H, R_2=COOEt, R_3=OBn
5: R_1=H, R_2=H, R_3=OH
8: R_1=H, R_2=Ph, R_3=OH
9: R_1=H, R_2=CH_3, R_3=OH
10: R_1=CH_3, R_2=CONHMe, R_3=OH
11: R_1=BOC, R_2=CH_3, R_3=OH
12: R_1=H, R_2=COOEt, R_3=O-i-C_3H_7
13: R_1=H, R_2=COOEt, R_3=O-n-C_4H_9
14: R_1=H, R_2=COOEt, R_3=OCH_3

N-hydroxytryptamine[3a] (*i.e.* **5**) have been investigated only incidentally. The preliminary results of these studies indicate a different behaviour with respect to the reactivity and stereochemistry. The chemical scope and the stereochemical implications of this reaction for N-hydroxy(alkoxy)-tryptophan and -tryptamine derivatives **3-5** and **8-14** (Chart I)[7] has now been studied in more detail with different aldehydes (R_4CHO).

Pictet-Spengler reactions

Reaction of the above mentioned N-hydroxy(alkoxy)-tryptophan and -tryptamine derivatives with aldehydes (R_4CHO) in dichloromethane at room temperature in the presence of trifluoroacetic acid (1

Table 1. Influence of the substituents (R_1-R_4) on the stereochemistry of the Pictet-Spengler reaction.



3-5, 8-14 **6** **7**

Entry	Product 6 or 7	R_1	R_2	R_3	R_4	Reaction Conditions	yield[a] (%)	Product ratio[b] 6/7
1	a[c]	H	COOEt	OH	CH_3	25°C, 5 h	98	70 / 30
2	b				$n\text{-}C_3H_7$	2 d	98	60 / 40
3	c				$CH_2C_6H_5$	2 d	98	58 / 42
4	d				$C_2H_4SCOCH_3$	1 h	99	71 / 29
5	e[c]				C_6H_5	3 h	85	43 / 57
6	f				2-thienyl	2 d	79	58 / 42
7	g				$3,4,5\text{-}C_6H_2(OMe)_3$	4 d	76	50 / 50
8	h	H	C_6H_5	OH	CH_3	25°C, 2h	94	70/30
9	i		CH_3			12h	87	86/14
10	j[e]		H			24h	83	-
11	k		C_6H_5		C_6H_6	3h	91	45/55
12	l		CH_3			40°C 24h	97	63/37
13	m		CH_3		$3,4,5\text{-}C_6H_2(OMe)_3$	40°C, 24h	97	66/34
14	n	CH_3	$CONHCH_3$	OH	C_6H_5	25°C, 3h	95	0/100
15		Boc	CH_3			competition between deprotection and condensation		
16	o	H	COOEt	OCH_3	CH_3	25°C, 1h	95	47/53
17	p[c]			$OCH_2C_6H_5$		3h	96	50/50[c]
18	q			O-i-C_3H_7		1h	80	42/58[d]
19	r			O-n-C_4H_9		1h	87	43/57[d]
20	s			OCH_3	C_6H_5	1h	97	18/82
21	t			O-i-C_3H_7		1h	96	21/79
22	u			O-n-C_4H_9		1h	93	25/75

a) based on isolated products b) based on isolated compounds c) see reference 3b d) product ratio determined by means of an analytical HPLC-technique e) see reference 3a

equiv.) gave a mixture of N-hydroxy(alkoxy)-1,2,3,4-tetrahydro-β-carbolines **6** and **7** (Table I). With the exception of entry 15 all variations of R_1-R_4 studied resulted in the desired β-carbolines in excellent yields. In order to establish the relative stereochemistry of the C(1) and C(3) protons, NOE

difference studies were carried out. Irradiation of C(1)H of compounds **6** resulted in a ca. 10% NOE on C(3)H and *vice versa*. The absence of these NOE differences in compounds **7** indicates that the C(1) and C(3) protons in **6** and in **7** have a *cis*- and a *trans*-relationship, respectively. It has been argued that proton NMR shifts cannot be used for the assignment of the stereochemistry of 1,3-disubstituted 1,2,3,4-tetrahydro-β-carbolines.[4a] We found that in all of the 1,3-disubstituted N-hydroxy(alkoxy)-β-carboline derivatives studied however, the chemical shifts of the C(1)H protons and the N-hydroxy protons of the 1,3-disubstituted N-hydroxy-β-carbolines of the *trans*-isomers (**7**) are consistently downfield (0.14-1.54 ppm) to the chemical shifts of the corresponding protons of the corresponding *cis*-isomers (**6**).

The influence of R_4.

The influence of the substituent R_4 on the relative stereochemistry (C(1)→(C(3)) was studied by the reaction of **3** with different aldehydes (Table I, Entries 1-7). The tendency is a selectivity for the *cis*-isomer **6**. Exceptions are the reactions with benzaldehyde and 3,4,5-trimethoxybenzaldehyde (entries 5 and 7). It has been reported[4g] that when R_3=H under reaction conditions similar to those using butyraldehyde or benzaldehyde, that the *cis* product is formed preponderantly (*cis/trans*=80/20). The decrease of this selectivity observed for N-hydroxytryptophan (R_3=OH, entries 2 and 5) is in agreement with results obtained for tryptophan derivatives in which R_3=alkyl.[4b,c,h]

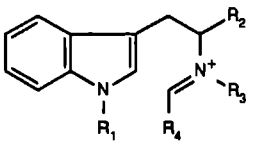
The observed stereochemistry seems to be the result of a kinetically controlled reaction. Prolonged treatment of either the *cis*-isomers **6b** and **6e** or the *trans*-isomers **7b** and **7e** respectively under the reaction conditions used for their formation did not cause the formation of the other isomer. Higher temperature (65°C) led to serious decomposition of the starting materials. In the resulting reaction mixtures the only other isomer could be detected, and in less than 5% yield by means of analytical HPLC techniques

Substituent R_2.

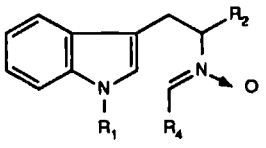
The influence of the α-substituent (R_2) on the reactivity and relative stereochemistry was studied by reaction of the N-hydroxy compounds **3**, **5** and **8-9** (R_2=COOEt, H, Ph and CH_3, respectively) with acetaldehyde and benzaldehyde derivatives (Table I, Entries, 1, 5 and 8-13). The substituents R_2=COOEt and R_2=Ph (Entries 1, 5, 8, 11) have a comparable influence on the stereochemistry, whereas R_2=CH_3 (Entries 9, 12, 13) causes a shift towards *cis*-selectivity. Based on the minimal reaction times mentioned in Table I, the reactivities of the compounds with R_2=COOEt (Entries 1 and 5) and R_2=Ph (Entries 8 and 11) are also comparable, whereas with R_2=H (Entry 10) and R_2=CH_3 (Entry 9) a decrease in reactivity is observed. The smooth reaction of N-hydroxytryptamine (**5**) with acetaldehyde (Entry 10[3a]) is surprising in view of the fact that tryptamine itself (R_3=H) cyclizes only under much more severe reaction conditions.[1b,4d] This may be rationalized as follows.

It is generally excepted that The Pictet-Spengler reaction involves the intermediacy of the imminium ion **15** (Chart II), and that the electrophilic character of the C=N bond in this intermediate explains differences in reactivity.[1b] Electron withdrawing groups (R_2 *e.g.* COOR) destabilize the imminium ion

Chart II



15



16

and accelerate the reaction. Similary the higher reactivity of N-hydroxytryptamine (R_3=OH) *versus* tryptamine (R_3=H) can be attributed to the electron-withdrawing ability of the hydroxy group. This increased reactivity is also reflected in a lower stereoselectivity.

Substituent R_1.

The influence of R_1 on the relative stereochemistry was studied by the reaction of the N-hydroxy compounds **10-11** with benzaldehyde (Table I, Entries 14-15). Examination of molecular models indicated that in the β-carboline **6** the $A_{(1,2)}$-strain[4a] between the substituents R_1 (CH_3 or BOC) and R_4 (Ph) will be so pronounced that by far mainly the *trans*-isomer **7** will be formed. Indeed, reaction of **10** (R_1=CH_3) with benzaldehyde gave a single diastereomer of which the relative stereochemistry was established as *trans* (Entry 14). On the basis of this observation, we reasoned that the introduction of a protective group at the indole nitrogen could lead to a highly stereoselective approach for *trans* 1,3-disubstituted N-hydroxy-1,2,3,4-tetrahydro-β-carbolines (**7**: R_1=H). Treatment of the N-BOC protected compound **11** with benzaldehyde under the acidic reaction conditions employed previously did not yield however the desired ring closed product. This process is so slow that N-deprotection then becomes a competitive reaction. This result can be rationalized by the decreased electron density of the indole C(2)-C(3) double bond due to the electron-withdrawing BOC group.

Substituent R_3.

Of special interest is the influence of R_3 on the relative stereochemistry. The reactions of N-alkoxytryptophan derivatives **4** and **12-14** with acetaldehyde and benzaldehyde (Table I, Entries 16-22) were studied. It is noteworthy that the N-alkoxytryptophans are more reactive than their N-hydroxy counterpart **3** (entries 1 and 5). This can be rationalized by when R_3=OH intermediate **15** is in equilibrium with the nitrone **16** (Chart II), and in the case of R_3=alkoxy such an equilibrium is not possible.

Another interesting feature is that the reaction with acetaldehyde, as well as with benzaldehyde, shows a shift of towards *trans* selectivity in going from NOH to NOR_3 derivatives (compare entries 1 with 16 and 5 with 20). In contrast with the complete *trans*-selectivity observed for the reaction of N-benzyltryptophan with benzaldehyde[4b], the N-alkoxytryptophans however showed no complete

selectivity. This can again be rationalized by the increased reactivity of intermediate **15** as a result of the electronic effect exerted by the oxygen atom.

Conclusions

In this study on the influence of the substituents R_1-R_4 on the course of the Pictet-Spengler reaction it was found that:

i) reactions of N-hydroxytryptophan **3** with aldehydes (R_4CHO) in general show a moderate selectivity for the *cis*-isomer.

ii) the reactivity and stereochemistry is influenced by the α-substituent R_2; compounds with R_2=COOEt or Ph are more reactive than compounds with R_2=H or CH_3; in the case of R_2=CH_3 the most pronounced *cis*-selectivity is observed.

iii) for R_3-substituents the reactivity and the *trans*-selectivity increase in the row H < OH < OR.

iv) the presence of a substituent other than H at the indole nitrogen (R_1) causes complete *trans*--selectivity.

Experimental Section

Melting points were taken on a Koefler hot stage (Leitz-Wetzlar) and are uncorrected. Ultraviolet spectra were measured with a Perkin Elmer spectrometer, Model lambda 5. Proton magnetic resonance spectra were measured on a Bruker WH-90 or on a Bruker AM 400 spectrometer. Chemical shifts are reported as δ-values relative to tetramethylsilane as an internal standard; deuteriochloroform was used as solvent unless stated otherwise. Mass spectra were obtained using a double-focusing VG 7070E spectrometer. Thin-layer chromatography (TLC) was carried out using silica gel F-254 plates (thickness 0.25 mm). Spots were visualized with a UV hand lamp, iodine vapor, or Cl_2-TDM.[8] For column chromatography Merck silica gel (type 60H) was used.

General procedure Pictet-Spengler reaction.

A stirred solution of a N-hydroxy(alkoxy)tryptophan or tryptamine derivative[7] (variation R_1-R_3, see Table I) (1 mmol) and aldehyde (R_4CHO, see Table I) (1.25 mmol) in dry dichloromethane (10 mL) was treated with trifluoroacetic acid (1 mmol) and the reaction allowed to proceed under the conditions given in Table I. The mixture was then concentrated *in vacuo* and the resultant residue subjected to column chromatography to yield compounds **6** and **7** (Table I). Spectroscopic data for these derivatives are recorded in Table II.

Table II Spectroscopic data of the β-carbolines 6 and 7

Prod.	mp (°C)	Rf (Solv Sys)[a]	Mass Spectrum	^{1}H NMR δ (ppm)
6b[b]	178-181	0 75 (D)	302 (M^+, 36), 285 ([M-OH]$^+$, 8), 259 ([M-C_3H_7]$^+$, 100), 229 ([M-COOEt]$^+$, 17), 185 ([$C_{11}H_9N_2O$]$^+$, 51), 169 ([$C_{11}H_9N_2$]$^+$, 42)	7 76 (br s, 1H,NH), 7 58-7 04 (m, 4H, C(5)-C(8)H), 5 64 (br s, 1H, NOH), 4 32 (q, 2H, OCH_2CH_3), 4 13 (br s, 1H, C(1)H), 3 86 (t, 1H, C(3)H), 3 10 (d, 2H, C(4)H_2), 2 22-1 53 (m, 4H, C(1)$CH_2CH_2CH_3$), 1 36 (t, 3H, OCH_2CH_3) 1 00 (t, 3H, C(1)$CH_2CH_2CH_3$)
7b[b]	141-144	0 45 (D)	302 (M^+, 46), 285 ([M-OH]$^+$, 86), 259 ([M-C_3H_7]$^+$, 100), 229 ([M-COOEt]$^+$, 29), 185 ([$C_{11}H_9N_2O$]$^+$, 45), 169 ([$C_{11}H_9N_2$]$^+$, 69)	7 62 (br s, 1H,NH), 7 47-6 89 (m, 4H, C(5)-C(8)H), 5 84 (br s, 1H, NOH), 4 27 (br t, 1H, C(1)H), 4 14 (q, 2H, OCH_2CH_3), 3 92 (X part of ABX spectrum, 1H, C(3)H), 3 11 and 2 97 (AB part of ABX spectrum, 2H, ^{2}J=15 4Hz, J=6 1 Hz, J-5 3 Hz, C(3)H), 1 96-1 49 (m, 4H, C(1)$CH_2CH_2CH_3$), 1 33 (t, 3H, OCH_2CH_3), 1 00 (t, 3H, C(1)$CH_2CH_2CH_3$)
6c[b]	160-162	0 71 (B)	350 (M^+, 5), 277 ([M COOEt]$^+$, 3), 259 ([M-C_7H_7]$^+$, 100), 169 ([$C_{11}H_9N_2$]$^+$, 35)	7 71-6 87 (m, 10H, NH, C(5)-C(8)H and C_6H_5), 6 11 (br s, 1H, NOH), 4 49 (br s, 1H, C(1)H), 4 32 (q, 2H, OCH_2CH_3), 4 00-3 64 (m, 3H, C(3)H and C(1)CH_2), 3 16-2 87 (m, 2H, C(4)H_2), 1 33 (t, 3H, OCH_2CH_3)
7c[b]	155-159	0 57 (B)	350 (M^+, 3), 333 ([M-OH]$^+$, 11), 277 ([M-COOEt]$^+$, 2), 259 ([M-C_7H_7]$^+$, 100), 169 ([$C_{11}H_9N_2$]$^+$, 39)	7 60-6 76 (m, 10H, NH, C(5)-C(8)H and C_6H_5), 6 40 (br s, 1H, NOH), 4 69 (X part of ABX spectrum, 1H, C(1)H), 4 27 (q, 2H, OCH_2CH_3), 4 13 (t, 1H, C(3)H), 3 67-2 49 (m, 4H, C(4)H_2 and AB part of ABX spectrum of C(1)HCH_2), 1 32 (t, 3H, OCH_2CH_3)
6d	foam[c]	0 74 (D)	362 (M^+, 30), 345 ([M-OH]$^+$, 18), 289 ([M-COOEt]$^+$, 7), 259 ([M-C_4H_7OS]$^+$, 100), 169 ([$C_{11}H_9N_2$]$^+$, 87)	8 73 (br s, 1H, NH), 7 44-7 07 (m, 4H, C(5)-C(8)H), 6 08 (br s, 1H, NOH), 4 29 (q, 2H, OCH_2CH_3), 4 12 (br s, 1H, C(1)H,), 3 82 (X part of ABX spectrum, 1H, C(3)H), 3 24-2.76 (m, 4H, C(4)H_2 and CH_2S), 2 31 (s, 3H, $SCOCH_3$), 2.17-1 78 (m, 2H, C(1)CH_2), 1 36 (t, 3H, OCH_2CH_3)
7d	foam[c]	0 67 (D)	362 (M^+, 25), 345 ([M-OH]$^+$, 63), 301 (37), 289 ([M-COOEt]$^+$, 9), 259 ([M-C_4H_7OS]$^+$, 74), 169 ([$C_{11}H_9N_2$]$^+$, 100)	8 57 (br s, 1H, NH), 7 52-7 00 (m, 4H, C(5)-C(8)H), 6 27 (br s, 1H, NOH), 4 44 (t, 1H, C(1)H), 4 21 (q, 2H, OCH_2CH_3), 4 06 (t, 1H, C(3)H), 3 41-2.80 (m, 4H, C(4)H_2 and CH_2S), 2 34 (s, 3H, $SCOCH_3$), 2 34-1 81 (m, 2H, C(1)CH_2), 1 27 (t, 3H, OCH_2CH_3)
6f[b]	168-170	0 69 (D)	342 (M^+, 7), 325 ([M-OH]$^+$, 9), 269 ([M-COOEt]$^+$, 11), 251 ([$C_{15}H_{11}N_2$]$^+$, 36), 225 ([$C_{14}H_{11}N$]$^+$, 100), 169 ([$C_{11}H_9N_2$]$^+$, 8)	7 61-6 80 (m, 8H, NH, C(5)-C(8)H and C_4H_3S), 5 83 (br s, 1H, NOH), 5 34 (br s, 1H, C(1)H), 4 42 (q, 2H, OCH_2CH_3), 4 00 (t, 1H, C(3)H), 3 20 (d, 2H, J=7 9Hz, C(4)H_2), 1 36 (t, 3H, OCH_2CH_3)
7f[b]	214-217	0 57 (D)	342 (M^+, 7), 325 ([M-OH]$^+$, 35), 269 ([M-COOEt]$^+$, 16), 251 ([$C_{15}H_{11}N_2$]$^+$, 71), 225 ([$C_{14}H_{11}N$]$^+$, 100), 169 ([$C_{11}H_9N_2$]$^+$, 14)	7 61-6 80 (m, 8H, NH, C(5)-C(8)H and C_4H_3S), 5 80 (s, 1H, C(1)H), 4 22 (q, 2H, OCH_2CH_3), 4 12 (t, 1H, C(3)H), 3 44 (m, 2H, C(4)H_2), 1 29 (t, 3H, OCH_2CH_3)
6g[b]	225-227	0 76 (D)	426 (M^+, 16), 409 ([M-OH]$^+$, 19), 353 ([M-COOEt]$^+$, 13), 335 (34), 309 ([$C_{19}H_{19}NO_3$]$^+$, 61), 278 ([$C_{18}H_{18}NO_2$]$^+$, 65), 219 (100), 169 ([$C_{11}H_9N_2$]$^+$, 9)	7 67 (br s, 1H, NH), 7 60-7 10 (m, 4H, C(5)-C(8)H), 6 73 (s, 2H, $C_6H_2(OMe)_3$), 5 50 (s, 1H, NOH), 4 95 (s, 1H, C(1)H), 4 33 (q, 2H, OCH_2CH_3), 4 04 (X part of ABX spectrum, 1H, C(3)H), 3 88 (s, 3H, p-(OCH_3)), 3 82 (s, 6H, 2x m-(OCH_3)), 3 55-3 11 (m, 2H, C(4)H_2), 1 34 (t, 3H, OCH_2CH_3)

a) A $CHCl_3$ B $CHCl_3$/MeOH, 99/1 C $CHCl_3$/MeOH, 97/3 D $CHCl_3$/MeOH, 93/7 b) Satisfactory micro analyses were obtained for these compounds (C±0 5%, H±0 2%, N±0 4%)

c) These products resisted crystallization attempts d) Attempts to separate these isomers failed

Table II Spectroscopic data of the β-carbolines **6** and **7**

Prod	mp (°C)	Rf (Solv Sys)[a]	Mass Spectrum	^{1}H NMR δ (ppm)
7g[b]	220-223	0 60 (D)	426 (M^+, 17), 409 ([M-OH]$^+$, 37), 353 ([M-COOEt]$^+$, 43), 309 ([$C_{19}H_{19}NO_3$]$^+$, 47), 278 ([$C_{16}H_{16}NO_2$]$^+$, 63), 219 (100), 169 ([$C_{11}H_9N_2$]$^+$, 27)	7 64 (br s, 1H, NH), 7 60-7 09 (m, 4H, C(5)-C(8)H), 6 62 (s, 2H, $C_6H_2(OMe)_3$), 6 03 (s, 1H, NOH), 5 69 (s, 1H, C(1)H), 4 40-4 07 (m, 3H, OCH_2CH_3 and C(3)H), 3 87 (s, 3H, p-(OCH_3)), 3 80 (s, 6H, 2x m-(OCH_3)), 3 44-3 14 (m, 2H, C(4)H_2), 1 28 (t, 3H, OCH_2CH_3)
6h[b]	101-103	0 10 (B)	278 (M^+, 20), 263 ([M-CH_3]$^+$, 2), 169 ([$C_{11}H_9N_2$]$^+$, 5), 159 (100)	7 73 (br s, 1H, NH), 7 61-6 96 (m, 9H, C(5)-C(8)H and C_6H_5), 4 73 (br s, 1H, NOH), 4 07 (br t, 2H, C(1)H and C(3)H), 3 04 (d, 2H, C(4)H_2), 1 62 (d, 3H, C(1)CH_3)
7h	foam[c]	0 05 (B)	278 (M^+, 21), 263 ([M-CH_3]$^+$, 7), 169 ([$C_{11}H_9N_2$]$^+$, 7), 159 (100)	7 78 (br s, 1H, NH), 7 63-7 02 (m, 9H, C(5)-C(8)H and C_6H_5), 5 02 (br s, 1H, NOH), 4 34 (q, 1H, C(1)H), 4 09 (X part of ABX soectrum, 1H, C(3)H), 3 38 and 3 08 (AB part of ABX spectrum, 2H, ^{2}J=14 9Hz, J=8 0 Hz, J=5 1Hz, C(4)H_2), 1 52 (d, 3H, C(1)CH_3)
6i	foam[c]	0 28 (D)	216 (M^+, 26), 157 ([$C_{11}H_{11}N$]$^+$, 100)	7 73 (br s, 1H, NH), 7 50-7 01 (m, 4H, C(5)-C(8)H), 5 08 (br s, 1H, NOH), 4 06 (br s, 1H, C(1)H), 3 14 (m, 1H, C(3)H), 2 74 (m, 2H C(4)H_2), 1 61 (d, 3H, C(1)CH_3), 1 42 (d, 3H, C(3)CH_3)
7i	foam[c]	0 22 (D)	216 (M^+, 25), 157 ([$C_{11}H_{11}N$]$^+$, 100)	7 70 (br s, 1H, NH), 7 52-7 06 (m, 4H, C(5)-C(8)H), 4 32 (q, 1H, C(1)H), 3 50 (m, 1H, C(3)H), 2 77 (m, 2H, C(4)H_2), 1 51 (d, 3H, C(1)CH_3), 1 32 (d, 3H, C(3)CH_3)
6j[b]	191-193	0 17 (C)	202 (M^+, 42), 157 ([$C_{11}H_{11}N$]$^+$, 100)	7 70 (br s, 1H, NH), 7 51-7 06 (m, 4H, C(5)-C(8)H), 6 47 (s, 1H, NOH), 4 03 (m, 1H, C(1)H), 3 55 (m, 1H, C(3)H_A), 3 20 (m, 1H, C(3)H_B), 2 97 (m, 2H, C(4)H_2), 1 59 (d, 3H, C(1)CH_3)
6k[b]	191-193	0 62 (B)	340 (M^+, 11), 219 ([$C_{16}H_{13}N$]$^+$, 100)	7 64-6 91 (m, 15H, NH, C(5)-C(8)H and 2xC_6H_5), 5 10 (br s, 1H, C(1)H), 4 59 (br s, 1H, NOH), 4 27 (t, 1H, J=7 5Hz, C(3)H), 3 16 (d, 2H, J=7 5Hz, C(4)H_2)
7k[b]	192-195	0 29 (B)	340 (M^+, 4), 219 ([$C_{16}H_{13}N$]$^+$, 100)	7 73-7 02 (m, 15H, NH, C(5)-C(8)H and 2xC_6H_5), 6 64 (br s, 1H, C(1)H), 4 84 (br s, 1H, NOH), 4 11 (X part of ABX spectrum, 1H, C(3)H), 3 42 and 3 14 (AB part of ABX spectrum, 2H, ^{2}J=15 6Hz,J=8 0Hz, J=4 8Hz, C(4)H_2)
6l[b]	95-97	0 39 (B)	278 (M^+, 20), 258 ([$C_{18}H_{14}N_2$]$^+$, 25), 245 ([$C_{17}H_{13}N_2$]$^+$, 23), 219 ([$C_{16}H_{13}N$]$^+$, 100)	7 53-7 02 (m, 10H, NH, C(5)-C(8)H and C_6H_5), 4 94 (s, 1H, C(1)H), 4 82 (br s, 1H, NOH), 3 30 (m, 1H, C(3)H), 2 86 (m, 2H, C(4)H_2), 1 47 (d, 3H, C(3)CH_3)

a) A $CHCl_3$ B $CHCl_3$/MeOH 99/1 C $CHCl_3$/MeOH, 97/3 D $CHCl_3$/MeOH, 93/7 b) Satisfactory micro analyses were obtained for these compounds (C±0 5%, H±0 2%, N±0 4%)
c) These products resisted crystallization attempts d) Attempts to separate these isomers failed

Table II Spectroscopic data of the β-carbolines **6** and **7**

Prod.	mp (°C)	Rf (Solv Sys)[a]	Mass Spectrum	^{1}H NMR δ (ppm)
7l[b]		0 19 (B)	278 (M^+ 24), 258 ($[C_{18}H_{14}N_2]^+$, 19), 245 ($[C_{17}H_{13}N_2]^+$, 24), 219 ($[C_{16}H_{13}N]^+$, 100)	7 59-7 08 (m, 10H, NH, C(5)-C(8)H and C_6H_5), 5 73 (br s, 1H, NOH), 5 18 (s, 1H, C(1)H), 3 56 (m, 1H, C(3)H), 3 03 and 2 81 (AB part of ABX spectrum, 2H, ^{2}J=15 5Hz, J=6 6Hz, J=8 5Hz, C(4)H_2), 1 29 (d, 3H, C(3)HCH_3)
6m[b]	230-232	0 62 (D)	368 (M^+, 24), 350 ($[M-H_2O]^+$, 43), 335 ($[C_{20}H_{19}N_2O_3]^+$, 57), 309 ($[C_{19}H_{19}NO_3]^+$, 100), 278 ($[C_{18}H_{16}NO_2]^+$, 94)	7 50-7 03 (m, 5H, NH and C(5)-C(8)H), 6 63 (s, 2H, C(1)C_6H_2(OMe)$_3$), 4 84 (br s, 2H, C(1)H and NOH), 3 89 (s, 3H, p-OCH_3), 3 83 (s, 6H, 2x m-OCH_3), 3 25 (m, 1H, C(3)H), 2 91 (m, 2H, C(4)H_2), 1 48 (d, 3H, C(3)HCH_3)
7m[b]	220-222	0 47 (D)	368 (M^+, 23), 350 ($[M-H_2O]^+$, 15), 335 ($[C_{20}H_{19}N_2O_3]^+$, 36), 309 ($[C_{19}H_{19}NO_3]^+$, 100), 278 ($[C_{18}H_{16}NO_2]^+$, 92)	7 56-7 06 (m, 5H, NH and C(5)-C(8)H), 6 54 (s, 2H, C(1)C_6H_2(OMe)$_3$), 5 50 (br s, 1H, NOH), 5 03 (s, 1H, C(1)H), 3 84 (s, 3H, p-OCH_3), 3 78 (s, 6H, 2x m-OCH_3), 3 64 (m, 1H, C(3)H), 3 11 and 2 78 (AB part of ABX spectrum, 2H, ^{2}J=15 5Hz, J=6 3Hz, J=4 5Hz, C(4)H_2), 1 29 (d, 3H, C(3)HCH_3)
7n[b]	211-223	0 26 (C)	335 (M^+, 8), 318 ($[M-OH]^+$ 46), 277 ($[M-CONHCH_3]^+$, 18), 259 ($[C_{18}H_{18}N_2]^+$, 100), 232 ($[C_{17}H_{14}N]^+$, 41)	7 70-7 10 (m, 4H, C(5)-C(8)H), 6 83 (br s, 1H, NHMe), 5 57 (s 1H, C(1)H), 5 26 (br s, 1H, NOH), 3 78-3 04 (m, 3H, C(3)H and C(4)H_2), 3 41 (s, 3H, indole N-CH_3), 2 78 (d, 3H, NHCH_3)
6o	oil[c]	0 46 (B)	288 (M^+, 22), 257 ($[M-OCH_3]^+$, 68), 215 ([M-COOEt]$^+$, 18), 183 ($[C_{12}H_{11}N_2]^+$, 68), 157 ($[C_{11}H_{11}N]^+$, 100)	7 73 (br s, 1H, NH), 7 48-7 05 (m, 4H, C(5)-C(8)H), 4 33 (q, 2H, OCH_2CH_3), 4 21 (m, 1H, C(1)H), 3 92-3 57 (m, 1H, C(3)H), 3 76 (s, 3H, $NOCH_3$), 3 40-2 92 (m, 2H, C(4)H_2), 1 64 (d, 3H, C(1)HCH_3), 1 39 (t, 3H, OCH_2CH_3)
7o	oil[c]	0 40 (B)	288 (M^+, 21), 257 ($[M-OCH_3]^+$, 10), 231 (22), 215 ([M-COOEt]$^+$, 16), 183 ($[C_{12}H_{11}N_2]^+$, 24), 157 ($[C_{11}H_{11}N]^+$, 100)	7 68 (br s, 1H, NH), 7 50-7 02 (m, 4H, C(5)-C(8)H), 4 64 (q, 1H, C(1)HCH_3), 4 22 (q, 2H, OCH_2CH_3), 4 09 (X part of ABX spectrum, 1H, C(3)H), 3 61 (s, 3H, $NOCH_3$), 3 22 and 3 10 (AB part of ABX spectrum, 2H, ^{2}J=16 0Hz, J=7 5Hz, J=6 2Hz, C(4)H_2), 1 49 (t, 3H, OCH_2CH_3)
6q[d] **7q**[d]	oil[c]	0 26 (B)	316 (M^+, 9), 257 ($[M-C_3H_7O]^+$, 51), 243 ([M-COOEt]$^+$, 11), 183 ($[C_{12}H_{11}N_2]^+$, 40), 157 ($[C_{11}H_{11}N]^+$, 100)	
6r[d] **7r**[d]	oil[c]	0 31 (B)	330 (M^+, 9), 257 ([M-COOEt and M-$C_4H_9O]^+$, 25), 183 ($[C_{12}H_{11}N_2]^+$, 18), 157 ($[C_{11}H_{11}N]^+$, 100)	
6s	oil[c]	0 76 (B)	350 (M^+, 12), 319 ($[M-OCH_3]^+$, 14), 277 ([M-COOEt]$^+$, 8), 245 ($[C_{17}H_{13}N_2]^+$, 12), 219 ($[C_{16}H_{13}N]^+$, 100)	7 50-7 06 (m, 10H, NH, C(5)-C(8)H and C_6H_5), 4 96 (s, 1H, C(1)H), 4 28 (q, 2H, OCH_2CH_3), 3 93 (X part of ABX spectrum, 1H, C(3)H), 3 30-2 96 (m, 2H, C(4)H_2), 3 08 (s, 3H, OCH_3), 1 33 (t, 3H, OCH_2CH_3)

a) A $CHCl_3$ B $CHCl_3$/MeOH, 99/1 C $CHCl_3$/MeOH, 97/3 D $CHCl_3$/MeOH, 93/7 b) Satisfactory micro analyses were obtained for these compounds (C±0 5%, H±0 2%, N±0 4%)
c) These products resisted crystallization attempts d) Attempts to separate these isomers failed

Table II. Spectroscopic data of the β-carbolines **6** and **7**

Prod.	mp (°C)	Rf (Solv Sys)[a]	Mass Spectrum	^{1}H NMR δ (ppm)
7s[b]	188-189	0 66 (B)	350 (M^+, 15), 319 ([M-OCH_3]$^+$, 68), 277 ([M-COOEt]$^+$, 21), 245 ([$C_{17}H_{13}N_2$]$^+$, 52), 219 ([$C_{16}H_{13}N$]$^+$, 96), 218 ([$C_{16}H_{12}N$]$^+$, 100)	7 58-7 07 (m, 10H, NH, C(5)-C(8)H and C_6H_5), 5 72 (s, 1H, C(1)H), 4 18 (q, 2H, OCH_2CH_3), 4 14 (t, 1H, C(3)H), 3 47 (s, 3H, OCH_3), 3 28 (d, 2H, C(4)H_2), 1 26 (t, 3H, OCH_2CH_3)
6t	oil[c]	0 54 (B)	378 (M^+, 14), 319 ([M-C_3H_7O]$^+$, 16), 305 ([M-COOEt]$^+$, 9), 245 ([$C_{17}H_{13}N_2$]$^+$, 17), 219 ([$C_{16}H_{13}N$]$^+$, 100)	7 52-7 04 (m, 10H, NH, C(5)-C(8)H and C_6H_5), 5 01 (s, 1H, C(1)H), 4 26 (q, 2H, OCH_2CH_3), 3 98 (X part of ABX spectrum, 1H, C(3)H), 3 61-2 96 (m, 3H, C(4)H_2 and $NOCHMe_2$), 1 36 (t, 3H, OCH_2CH_3), 0 87 (d, 3H, J=6 1Hz, $OCH(CH_3)_A(CH_3)_B$), 0 44 (d, 3H, J=6 1Hz, $OCH(CH_3)_A(CH_3)_B$)
7t[b]	151-153	0 46 (B)	378 (M^+, 16), 319 ([M-C_3H_7O]$^+$, 61), 305 ([M-COOEt]$^+$, 27), 245 ([$C_{17}H_{13}N_2$]$^+$, 48), 219 ([$C_{16}H_{13}N$]$^+$, 100)	7 56-7 03 (m, 10H, NH, C(5)-C(8)H and C_6H_5), 5 73 (s, 1H, C(1)H), 4 16 (t, 1H, C(3)H), 4 14 (q, 2H, OCH_2CH_3), 3 64 (m, 1H, $NOCHMe_2$), 3 28 (d, 2H, J=5 8Hz, C(4)H_2), 1 26 (t, 3H, OCH_2CH_3), 1 08 (d, 3H, J=6 1Hz, $OCH(CH_3)_A(CH_3)_B$), 0 87 (d, 3H, J=6 1Hz, $OCH(CH_3)_A(CH_3)_B$)
6u	oil[c]	0 64 (A)	392 (M^+, 9), 319 ([M COOEt and M-C_4H_9O]$^+$, 18), 245 ([$C_{17}H_{13}N_2$]$^+$, 13), 219 ([$C_{16}H_{13}N$]$^+$, 100)	7 55-7/04 (m, 10H, NH, C(5)-C(8)H and C_6H_5), 5 00 (s, 1H, C(1)H), 4 28 (q, 2H, OCH_2CH_3), 3 97 (X part of ABX spectrum, 1H, C(3)H), 3 74-3 42 (m, 2H, $NOCH_2$), 3 40-2 71 (m, 2H, C(4)H_2), 1 37 (t, 3H, OCH_2CH_3), 1 21-0 83 (m, 4H, $OCH_2CH_2CH_2CH_3$), 0 69 (br t, 3H, $OCH_2CH_2CH_2CH_3$)
7u	oil[c]	0 53 (A)	392 (M^+, 12), 319 ([M-COOEt and M-C_4H_9O]$^+$, 72), 245 ([$C_{17}H_{13}N_2$]$^+$, 43), 219 ([$C_{16}H_{13}N$]$^+$, 100)	7 56-7 08 (m, 10H, NH, C(5)-C(8)H and C_6H_5), 5 76 (s, 1H, C(1)H), 4 19 (t, 1H, C(3)H), 4 15 (q, 2H, OCH_2CH_3), 3 78-3 41 (m, 2H, $NOCH_2$), 3 29 (d, 2H, J=6 0Hz, C(4)H_2), 1 47-1 00 (m, 4H, $OCH_2CH_2CH_2CH_3$), 1 26 (t, 3H, OCH_2CH_3), 0 79 (br t, 3H, $OCH_2CH_2CH_2CH_3$)

a) A $CHCl_3$ B $CHCl_3$/MeOH, 99/1 C $CHCl_3$/MeOH, 97/3 D $CHCl_3$/MeOH, 93/7 b) Satisfactory micro analyses were obtained for these compounds (C±0 5%, H±0 2%, N±0 4%)

c) These products resisted crystallization attempts d) Attempts to separate these isomers failed

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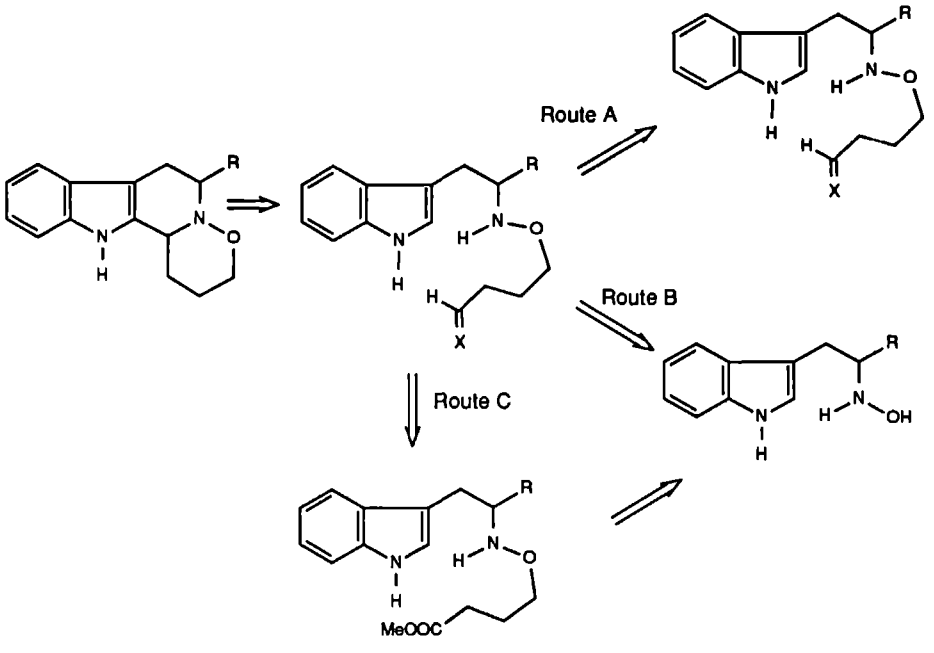
CHAPTER 6

Intramolecular Pictet-Spengler reaction of N-alkoxy--tryptophans and -tryptamines. Synthesis of Corynanthe alkaloid derivatives containing a tetrahydro-1,2-oxazine as D-ring.



"Intramolecular Pictet-Spengler reaction of N-alkoxytryptophans and tryptamines II. Synthesis of Corynanthe alkaloid derivatives containing a tetrahydro-1,2-oxazine as D-ring."
Pedro H.H. Hermkens, Jan H.v. Maarseveen, Harry W. Berens, Jan M.M. Smits, Chris G. Kruse, Hans W. Scheeren, *J. Org. Chem.* in press.

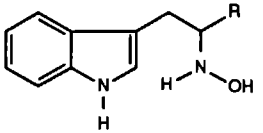
Chapter 6

INTRAMOLECULAR PICTET-SPENGLER REACTION OF N-ALKOXY-TRYPTOPHANS AND -TRYPTAMINES. SYNTHESIS OF CORYNANTHE ALKALOID DERIVATIVES CONTAINING A TETRAHYDRO-1,2-OXAZINE AS D-RING

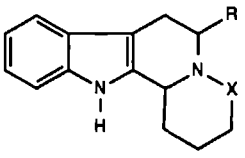
Introduction

In chapter 5 we described the chemical scope and the stereochemical implications of the intermolecular Pictet-Spengler reaction of N-hydroxy(alkoxy)-tryptophan and -tryptamine derivatives (*e.g.* **1-3**, Chart I) with different aldehydes to give the tricyclic N-hydroxy(alkoxy)-1,2,3,4-tetrahydro-β-carbolines[1]. The therapeutic activity of several tetra- or pentacyclic indole alkaloids which contain the tetrahydro-β-carboline nucleus has been demonstrated, *e.g.* for the corynantheine, and yohimbine (reserpine) group[2], respectively. We thought that the N-oxo analogues of these indole alkaloids

Chart I



1 R=COOEt
2 R=H
3 R=CH_3



4 R=COOEt, X=O
5 R=H, X=O
6 R=CH_3, X=O
7 R=COOMe, X=CH_2
8 R=H, X=CH_2

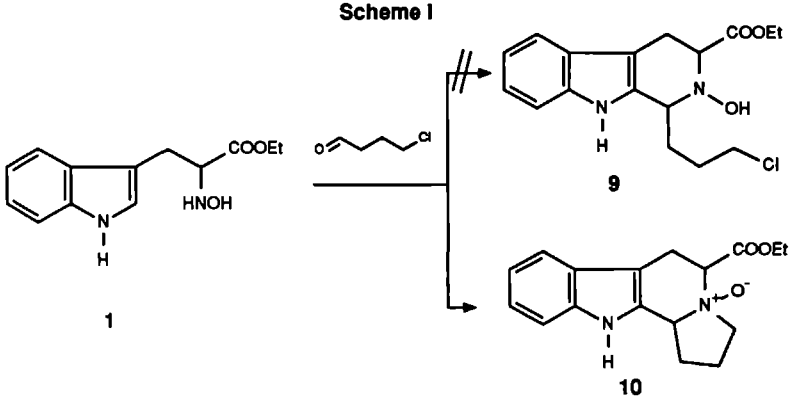
might be of potential interest. The first target structures we settled upon are **4-6** since they contain the structural elements of the N-hydroxy compounds **1-3**. These molecules are direct analogs of the simple corynanthe alkaloids **7**[3e,4] and **8**[3,5], containing a tetrahydro-1,2-oxazine as D-ring.

Strategy

The standard methods for the construction of the C-ring in the corynanthe alkaloids are the Bischler-Napieralski[6b] and the Pictet-Spengler[6b] reaction or cyclization via pyridinium salts[6b,7]. Recently, the conversion of **1** into 1,3-disubstituted N(2)-hydroxy-1,2,3,4-tetrahydro-β-carbolines via the Pictet-Spengler reaction has been reported[1,8]. Therefore our first approach to the compounds **4-6** consisted of a Pictet-Spengler reaction with 4-chlorobutanal, followed by a cylization to form ring D (Scheme I). However, condensation of N-hydroxytryptophan (**1**) with 4-chlorobutanal did not give the desired compound **9** but the N-oxide **10**, which has probably been formed by an undesired intramolecular cyclization of **9**.[9](Scheme I).

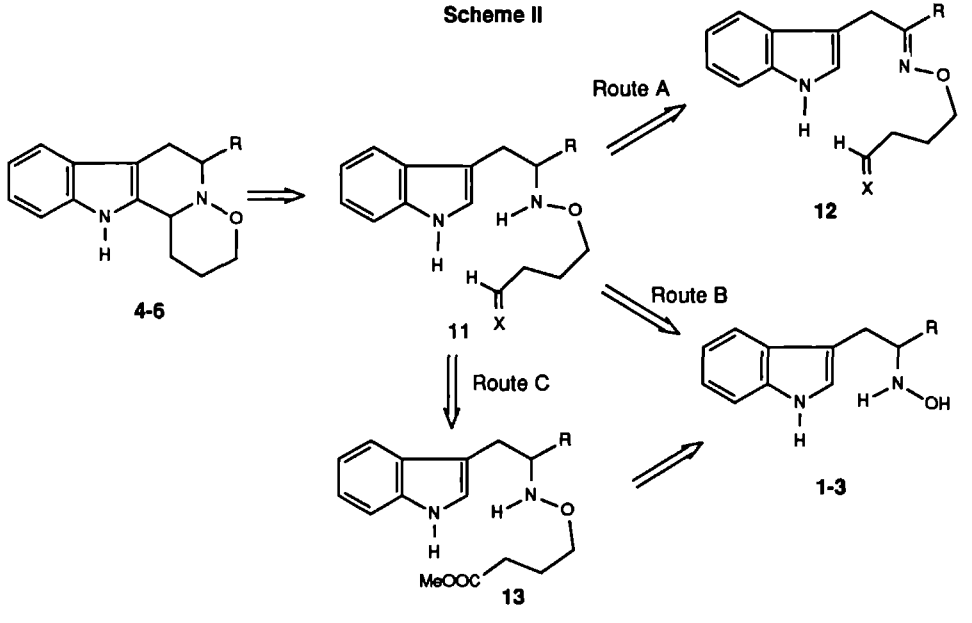
We reasoned that an intramolecular Pictet-Spengler reaction should be a better approach to the target

Scheme I



molecules. The intermediate for this synthetic pathway to compounds **4-6**, consists of a N-alkoxytryptamine derivative such as **11** having a aldehyde function or a masked derivative thereoff in δ-position of the alkoxy chain (Scheme II). Three routes as depicted in Scheme II might give access

Scheme II



to such an intermediate: (1) selective reduction of the O-alkylated oxime function present in **12** (Route A) (2) selective O-alkylation of the N-hydroxy compounds **1-3** with a properly functionalized four carbon substrate (Route B) or (3) selective reduction of the ester function in **13**, generated from **1-3** (Route C)[10].

In this chapter it is highlighted that compounds with the general formula **11** are highly valuable

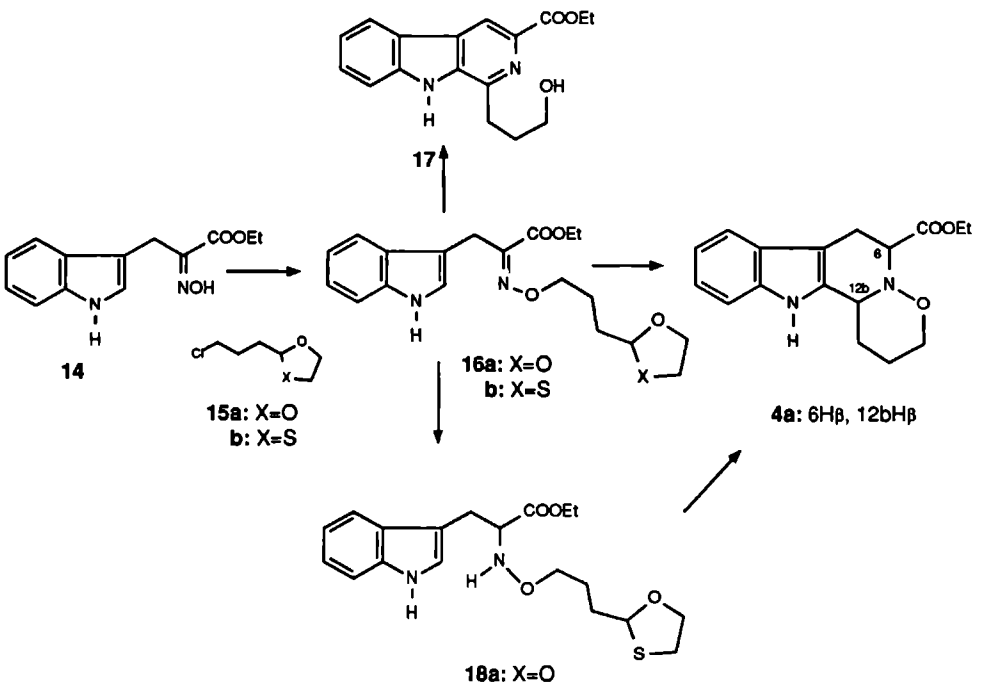
intermediates for the corynanthe N_b-oxo-analogous **4-6**, via an intramolecular Pictet-Spengler condensation. The most effective approaches are routes B and C, whereas route A gave poor results.

Results

Route A

Alkylation of oxime **14** with the protected 4-chlorobutanals **15** in DMSO with potassium t-butoxide as base gave the O-alkylated products **16** in 64-68% yields (Scheme III). Selective reduction of the

Scheme III



oxime double bond of the dioxolane protected compound **16a** failed. Treatment with trimethylamineborane in dioxane saturated with HCl gave a mixture of compounds. One of the products appeared to be the desired **4a**[11] presumably as a result of reduction of the oxime double bond[12] and intramolecular cyclization under the acidic conditions. The yield however was too low (10%)[13] to be of practical value as a result of side-reactions due to cationic ring opening of the dioxolane ring. This ring opening was followed by *i)* reduction of the dioxolane functionality (24%) to give a mono-glycolether moiety, partly accompanied by reduction of the oxime function *ii)* probably intramolecular cyclization to give a tetracyclic dihydro-β-carboline, followed by ring opening of the D-ring resulted in the aromatized product **17** (16%). Reaction of **16a** under the same reaction

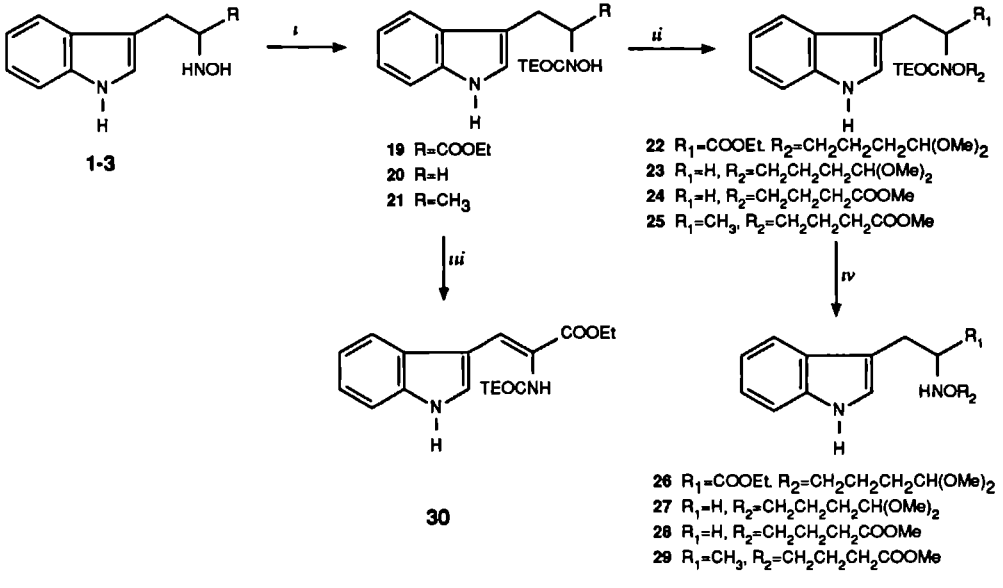
conditions with exclusion of the reductive reagent gave **17** quantitatively.

Reduction of the oxime double bond of the 1,3-oxathiolane protected compound **16b** with TMA:BH_3 in ethanolic HCl gave the N-alkoxy tryptophan derivative **18b** in 56% yield. In this case the previous described intramolecular cyclization was not observed. However, deprotection of the oxathiolane moiety of **18b** failed; the conditions studied were treatment with $HgCl_2$/NaOH[14b], Raney nickel[14a,b] and various acids. Deprotection with Chloramine-T[14c] gave the cyclized product **4a** but only in 22% yield. Attempts to improve these results were unsuccessful.

Routes B and C

In both routes B and C we faced the problem of selective O-alkylation of the N-substituted hydroxylamines **1-3**. In general N,O-disubstituted hydroxylamines are prepared by alkylation of N-hydroxyurethanes followed by acidic hydrolysis.[15] By adjusting the protective group this method could be suitable for our goal. The protective group has to be easily incorporated and removed and has to survive the alkylation conditions. For example, the trichloroethoxycarbonyl (TrOC) group satisfied

Scheme IV



i) TEOC-Cl / dioxane ii) K_2CO_3, R_2Cl/DMSO (R=COOEt) or NaH, RBr/NaI/DME (R=H,CH_3) iii) KOtBu / DMSO iv) Bu_4NF

the first two criteria, but failed with respect to the last one. The protective group of choice which met all the criteria is the 2-(trimethylsilyl)ethoxycarbonyl[16] (TEOC) group. Treatment of the hydroxylamines **1-3** with 2-(trimethylsilyl)ethyl chloroformate in dichloromethane / dioxane[17] at room temperature gave **19, 20** and **21** in 96%, 91% and 80% yield, respectively (Scheme IV).

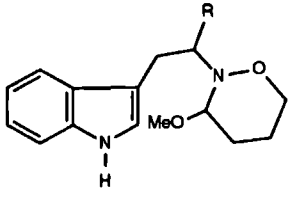
The optimal reaction conditions for the subsequent O-alkylation of these TEOC-protected

compounds depend on the α-substituent R in the tryptamine moiety. An N-acyl-N-hydroxytryptophan derivative such as **19** is sensitive to elimination reactions under basic conditions. In the absence of a nucleophile, rearrangements to the corresponding enamine ester derivative have been reported[18,19]. It occurred indeed that alkylation attempts with 4-bromo-1,1-dimethoxybutane employing **19** using DMSO/KOtBU or DME/NaH yielded the dehydro ester **30** almost quantitatively (Scheme IV). However, with K_2CO_3 as the base in DMSO at 45°C the desired **22** was obtained in 67% yield. The TEOC-protective group was removed with tetrabutylammoniumfluoride (Bu_4NF) in THF to give the N-alkoxytryptophan **26** in 78% yield. Since **20** and **21** are less prone to this elimination reaction these compounds could be smoothly converted at room temperature in DME with functionalized alkylbromides in the presence of NaI using NaH as the base to give **23-25**. These compounds were not purified, but immediately deprotected with Bu_4NF in THF yielding the compounds **27-29** in an overall yield of 67%, 74% and 64%, respectively.

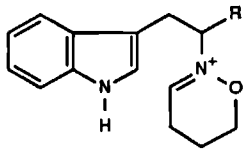
Cyclization (Scheme V)

It has been demonstrated that dimethoxy acetals react easily with **1** in the presence of trifluoroacetic acid to give N-hydroxy-1,2,3,4-tetrahydro-β-carbolines.[8] So by preparing the compounds **26** and **27**, which bear all necessary features of intermediate **11**, route B seems to be feasible. Indeed, treatment of **26** and **27** with trifluoroacetic acid in dichloromethane caused an intramolecular Pictet-Spengler reaction and presumably via intermediates **31a,b→32a,b** (Chart II) the cyclized products **4** and **5** were

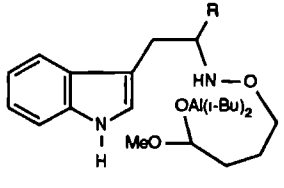
Chart II



31a: R=COOEt
31b: R=H
31c: R=CH_3



32a: R=COOEt
32b: R=H
32c: R=CH_3

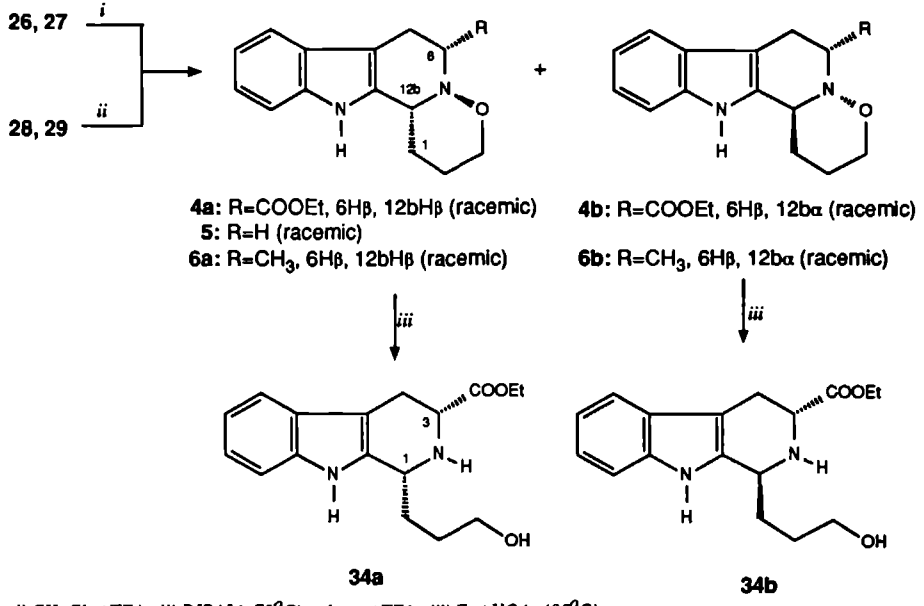


33a: R=H
33b: R=CH_3

isolated in 97% and 92% yield, respectively. The corynanthe analogue **4** was obtained as a 79/21 **(4a/4b)** mixture of the two possible diastereomers, which were readily separated by column chromatography. The relative stereochemistry at the C(6) and C(12b) centers were established as **4a** *cis* and **4b** *trans* (Scheme V) (*vide infra*).

In order to enlarge the scope of this intramolecular Pictet-Spengler reaction we also studied Route C. This route implies the selective reduction of the ester function in **28** and **29** in the presence of the labile N-O bond. Therefore, we were pleased to find that reduction of the methylester function of **28** with DIBAL in toluene at -70°C, followed by addition of trifluoroacetic acid gave the cyclised product **5** in 76% yield. Under the anhydrous acidic conditions intermediate **33a** cyclized either via **31b→32b→5** or via **11** (R=H, X=O)→**32→5** (Chart II).

Scheme V



Reduction of **29** employing the same conditions gave the two diastereomers **6a** and **6b** (HPLC ratio 83/17). Separated by column chromatography gave **6a** in 68% and **6b** in 9% yield. The stereochemistry was assigned by extending the line of reasoning employed for **4a** and **4b**: the major isomer was assumed to be the *cis* diastereomer **6a** given that the predominate isomer in the cyclization of **26** was unambiguously demonstrated to be **4a** (*cis*) (*vide infra*).

The observed stereochemistry seems to be the result of a kinetically controlled reaction. Prolonged treatment of either the *cis*-isomers **4a** and **6a** or the *trans*-isomers **4b** and **6b**, respectively under the reaction conditions caused no formation of the other isomer. Higher temperature (65°C) led to extensive decomposition of the starting materials. In the reaction mixtures the other isomer could be only detected in less than 5% by means of analytic HPLC techniques

Stereochemistry

The relative configuration of the two diastereomers **4a** and **4b** was established as follows. Treatment of **4a** and **4b** with zink dust in acetic acid at elevated temperature (80°C) gave the 1,3-disubtituted tetrahydro-β-carbolines **34a** and **34b** in 85% and 89% yield, respectively (Scheme V). Their relative stereochemistry was assigned on basis of ^{13}C NMR data. It has been noted[20] that in the off-resonance-decoupled ^{13}C NMR spectra of *trans*-1,3-disubstituted-1,2,3,4-tetrahydro-β-carbolines the chemical shift values for the C(1) and C(3) atoms are upfield of the values of the corresponding C-atoms in the *cis*-isomer. Consequently, structure **34b** was assigned *trans* because of its more

shielded C(3) (δ=51.90) and C(1) (δ=50.30) carbon atoms, compared to C(3) (δ=56.38) and C(1) (δ=52.60) of **34a**. Thus the relative stereochemistry at C(12b) and C(6) of the two diastereomers **4a** and **4b** is as depicted in Scheme V. Recently, Rapoport[4e] reported that the cyclization yielding the corynanthe alkaloid **8** via an *in situ* generated iminium ion gave the corresponding diastereomers in the same ratio as we observed for the reaction leading to **4a** and **4b**. So it seems to be reasonable to suppose that cylization of **26** proceeds via the *in situ* generation of the intermediate **32a**.

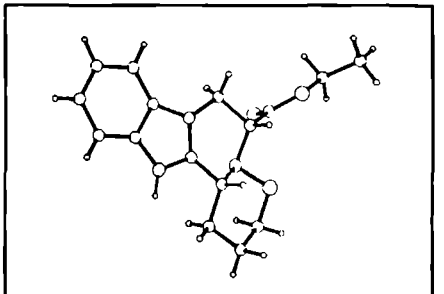


Figure I. PLUTO drawing of **4a**.

The above discussed assignment of stereochemistry could be confirmed by single crystal X-ray analysis of **4a** (Figure I).[21] The stereochemistry at N(5) is noteworthy. The crystal structure clearly shows that the tetrahydro-1,2-oxazine ring D has a chair conformation with the carbon substituents on N(5) and C(12b) in an equatorial position. This implies a *trans* juncture of ring C and D as depicted in Scheme V. The ethylester at C(6) is also in an equitorial position.

Conclusions

In conclusion, corynanthe analogs **4-6** have been synthesized by an intramolecular Pictet-Spengler reaction of **26-29** in excellent yields (Route B and C). The cyclizations occur with high stereoselectivity favouring the product with a relative *cis* configuration of the C(6) and C(12b) substituents. In general, this method seems to be appropriate for constructing tetracylic indole alkaloids of which the D-ring contains an N-O moiety. Therefore, the natural product *Eudistomin*[22], an indole alkaloid with a oxathiazepine as D-ring, will be our next target structure in order to test the general applicability of the intramolecular Pictet-Spengler approach (See chapter 7).

Experimental Section

Melting points were taken on a Koefler hot stage (Leitz-Wetzlar) and are uncorrected. Ultraviolet spectra were measured with a Perkin Elmer spectrometer, Model lambda 5. Proton magnetic resonance spectra were measured on a Bruker WH-90 spectrometer or on a Bruker AM 400. Chemical shifts are reported as δ-values relative to tetramethylsilane as an internal standard; deuteriochloroform was used as solvent unless stated otherwise. Mass spectra were obtained with a double-focusing VG 7070E spectrometer. Thin-layer chromatography (TLC) was carried out by using silica gel F-254 plates (thickness 0.25 mm). Spots were visualized with a UV hand lamp, iodine vapor, or Cl_2-TDM.[23] For column chromatography Merck silica gel (type 60H) was used. Solvent systems used are, A: ($MeOH/CHCl_3$, 3/97, v/v), B:($MeOH/CHCl_3$, 7/93, v/v), C:(EtOAc/n-hexane, 25/75, v/v) D:(EtOAc/n-hexane, 40/60, v/v).

Ethyl α-[3-(1,3-dioxolan-2-yl)propyloximino]-β-(indol-3-yl)propanoate (16a)

Potassium t-butoxide (0.74 g, 6.6 mmol) was added portionwise to a stirred solution of **14**[18] (1.63 g, 6.6 mmol) and **15a**[24] (1 g, 6.6. mmol) in DMSO (25 mL). After stirring for 2 hours at 50°C the reaction was complete. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and most of the DMSO was removed by washing three times with 100 mL of water. The resulting organic layer was washed with brine and dried with Na_2SO_4. Evaporation of the solvent gave an oil which was subjected to column chromatography ($CHCl_3$) to give 1.52 g (64%) **16a**. Oil: Rf 0.62 (solvent system A); UV (MeOH) λmax 224, 274(sh), 281, 289 nm; CIMS (100eV) m/z (relative intensity) 361 ($[M+1]^+$, 30), 360 (M^+, 33), 229 ($[C_{13}H_{13}N_2O_2]^+$, 26), 130 ($[C_9H_8N]^+$, 100); ^1H NMR δ 8.08 (br s, 1H, NH), 7.76-7.04 (m, 5H, indole C(2)H and C(4)-C(7)H), 4.91 (t, 1H, OCHO), 4.32 (m, 4H, OCH_2CH_3 and $NOCH_2$), 4.07 (s, 2H, C(3)CH_2), 4.00-3.82 (m, 4H, OCH_2CH_2O), 2.04-1.71 (m, 4H, CH_2CH_2CH), 1.27 (t, 3H, OCH_2CH_3).

Ethyl α-[3-(1,3-oxathiolan-2-yl)propyloximino]-β-(indol-3-yl)propanoate (16b)

The same procedure was followed as described for **16a**. **14**[18] (10 g, 41 mmol), Potassium t-butoxide (4.6 g, 41 mmol) and **15b**[25] (10 g, 60 mmol) in DMSO (100 mL) gave after column chromatography ($CHCl_3$) 10.5 g (68%) **16b** as an oil. Rf 0.76 (solvent system A); UV (MeOH) λmax 224, 274(sh), 281, 289 nm; CIMS (100eV) m/z (relative intensity) 376 (M^+, 6), 230 ($[C_{13}H_{14}N_2O_2]^+$, 16), 144 ($[C_{10}H_{10}N]^+$, 48), 130 ($[C_9H_8N]^+$, 100); ^1H NMR δ 8.11 (br s, 1H, NH), 7.64-7.00 (m, 5H, indole C(2)H and C(4)-C(7)H), 5.03 (br t, 1H, OCHS), 4.38-4.11 (m, 5H, OCH_2CH_3, $NOCH_2$ and $SCHOCH_A$), 4.02 (s, 2H, C(3)CH_2), 3.87-3.51 (m, 1H, $SCHOCH_B$), 3.08-2.87 (m, 2H, CH_2S), 2.00-1.70 (m, 4H, CH_2CH_2CH), 1.23 (t, 3H, OCH_2CH_3).

1-(3-Hydroxypropyl)-3-ethoxycarbonyl-β-carboline (17)

Through a stirred solution of **16a** (150 mg, 0.42 mmol) in THF (10 mL) was passed a stream of HCl for 2 minutes. Stirring was continued for 4 hours. The reaction mixture was washed with saturated $NaHCO_3$ and brine. The organic layer was dried ($MgSO_4$) and concentrated in vacuo. The residue was subjected to column chromatography (solvent system A) to give 120 mg (96%) of **17**. Crystallization attempts were unsuccessful. Rf 0.17 (solvent system B); EIMS (70 eV) m/z (relative intensity) 298 (M^+, 1), 254 ($[C_{15}H_{14}N_2O_2]^+$, 19), 169 ($C_{11}H_9N_2]^+$, 14), 69 (100); ^1H NMR ($CDCl_3/CD_3OD$) δ 11.14 (br s, 1H, NH exchangeable), 8.71 (s, 1H, C(4)H), 8.14 (d, 1H, C(5)H), 7.62-7.22 (m, 3H, C(6)-C(8)H), 4.51 (q, 2H, OCH_2CH_3), 3.76 (t, 2H, CH_2OH), 3.30 (t, 2H, C(1)CH_2), 2.24-1.98 (m, 2H, $CH_2CH_2CH_2OH$), 1.51 (t, 3H, OCH_2CH_3).

Ethyl α-[3-(1,3-oxathiolan-2-yl)propyloxamino]-β-(indol-3-yl)propanoate (18b)

A solution of HCl in ethanol (2 mL, 7N solution) was added dropwise to a stirred solution of **16b** (0.5 g, 1.3 mmol) and $(CH_3)_3N.BH_3$ (Aldrich Chemical Co; 300 mg, 4.16 mmol) in EtOH (10 mL) at roomtemperature and in an argon atmosphere. Stirring was continued for 5 h. The mixture was then concentrated to near dryness. The residue dissolved in CH_2Cl_2. This solution was neutralized with $NaHCO_3$ and filtered. The filtrate was washed with 0.1 N HCl and dried over Na_2SO_4. Evaporation of the solvent gave an oil which after column chromatography ($CHCl_3$) yielded 273 mg (56%) of **18**: oil; Rf 0.57 (solvent system A); UV (MeOH) λmax 224, 274(sh), 281, 289 nm; EIMS (70eV) m/z (relative intensity) 378 (M^+, 2), 305 ($[M-COOEt]^+$, 4), 227 ($[C_{14}H_{15}N_2O]^+$, 18), 130 ($[C_9H_8N]^+$, 100); ^1H NMR δ 8.11 (br s, 1H, NH), 7.67-7.04 (m, 5H, indole C(2)H and C(4)-C(7)H), 5.82 (br s, 1H, HNO), 5.07 (br t, 1H, OCHS), 4.40-3.90 (m, 4H, OCH_2CH_3, HCCOOEt and $SCHOCH_A$), 3.88-3.60 (m, 3H, $NOCH_2$ and $SCHOCH_B$), 3.12-2.92 (m, 4H, C(3)CH_2 and CH_2S), 2.02-1.55 (m, 4H, CH_2CH_2CH), 1.17 (t, 3H, OCH_2CH_3).

Ethyl α-[N,N-(2-(trimethylsilyl)ethyloxycarbonyl, hydroxy)amino]-β-(indol-3-yl)propanoate (19)

2-(Trimethylsilyl)ethylchloroformate[15] (1.08 g, 6 mmol) was added dropwise to a stirred solution of **1**[1] (1.0 g, 4 mmol) in CH_2Cl_2/dioxane, 1/1, v/v (25 mL). The reaction was monitored by TLC (solvent system B). Stirring was continued for 2 hours. The reaction mixture was concentrated to near dryness, dissolved in CH_2Cl_2 and subsequently washed with saturated $NaHCO_3$ and brine and dried with Na_2SO_4. Evaporation of the solvent gave a crystalline material which was subjected to column chromatography ($CHCl_3$/n-hexane, 99.5/0.5, v/v) to yield 1.51 g. (96%) of **19**. Crystallization from CH_2Cl_2/n-hexane: mp 101-102.5 °C; Rf 0.45 (solvent system A); UV (MeOH) λmax 224, 274(sh), 281, 289 nm; CIMS (100 eV) m/z (relative intensity) 393 ($[M+1]^+$, 9), 392 (M^+, 23), 365 (28), 349

(49), 321 (27), 247 ([M-$C_6H_{13}O_2Si$]$^+$, 11), 231 (53), 216 (67), 215 (95), 130 ([C_9H_8N]$^+$, 100);^{1}H NMR δ 8.23 (br s, 1H, NH), 7.77-7.19 (m, 5H, indole C(2) and C(4)-C(7)H), 6.53 (br s, 1H, NOH), 5.11 (t, 1H, J=7.8 Hz, HCCOOEt), 4.37 (q, 2H, OCH_2CH_3), 3.91 (m, 2H, OCH_2CH_2Si), 3.51 (d, 2H, J=7.8 Hz, Indole C(3)CH_2), 1.39 (t, 3H, OCH_2CH_3), 0.71 (m, 2H, CH_2Si), 0.0 (s, 9H, $Si(CH_3)_3$); Anal.Calcd. for $C_{19}H_{28}N_2O_5Si$ (Mw 392.531): C, 58.14; H, 7.19; N, 7.14. Found: C, 57.83; H, 7.14; N, 7.16.

3-[2-(N,N-2-(Trimethylsilyl)ethyloxycarbonyl, hydroxy)aminoethyl]indole (20)

The same procedure was followed as described for **19**. 2-(Trimethylsilyl)ethylchloroformate[15] (675 mg, 3.75 mmol) and **2**[1,26] (440 mg, 2.5 mmol) gave after column chromatography (EtOAc/n-hexane, 40/60, v/v) 750 mg (91%) of **20.** Crystallization from EtOAc/n-hexane: mp 95-97°C; Rf 0.39 (solvent system B); UV (MeOH) λmax 224, 274(sh), 281, 289 nm; EIMS (70 eV) m/z (relative intensity) 320 (M^+, 1), 157 ([$C_{10}H_9N_2$]$^+$, 11), 130 ([C_9H_8N]$^+$, 100); ^{1}H NMR δ 8.02 (br s, 1H, NH), 7.70-7.04 (m, 5H, indole C(2) and C(4)-C(7)H), 6.25 (br s, 1H, NOH), 4.11-3.87 (m, 2H, OCH_2CH_2Si), 3.90 (t, 2H, CH_2N), 3.13 (t, 2H, Indole C(3)CH_2), 0.89-0.68 (m, 2H, CH_2Si), 0.0 (s, 9H, $Si(CH_3)_3$); Anal.Calcd. for $C_{16}H_{24}N_2O_3Si$ (Mw 320469): C, 59.97; H, 7.55; N, 8.74. Found: C,59.91; H,7.62; N, 8.70.

3-[2-(N,N-2-(Trimethylsilyl)ethyloxycarbonyl, hydroxy)aminopropyl]indole (21)

The same procedure was followed as described for **19**. 2-(Trimethylsilyl)ethylchloroformate[15] (1.63 g, 9 mmol) and **3**[1,26] (1.14 g, 6 mmol) gave after column chromatography ($CHCl_3$/n-hexane, 99/1, v/v) 1.61 g (80%) of **21.** Crystallization from $CHCl_3$/n-hexane: mp 122-125 °C; Rf 0.29 (solvent system B); UV (MeOH) λmax 224, 274(sh), 281, 289 nm; CIMS (100 eV) m/z (relative intensity) 334 (M^+, 2), 291 (14), 230 (9), 158 ([$C_{11}H_{12}N$]$^+$, 100), 130 ([C_9H_8N]$^+$, 85), 73 ([$Si(CH_3)_3$]$^+$, 100); ^{1}H NMR δ 8.07 (br s, 1H, NH), 7.68-7.07 (m, 5H, indole C(2) and C(4)-C(7)H), 6.20 (br s, 1H, NOH), 4.53 (m, 1H, $HCCH_3$), 3.84 (m, 2H, OCH_2CH_2Si), 3.18 and 2.94 (AB part of ABX spectrum, 2H, ^{2}J=14.3 Hz, J=8.1 Hz, J=6.0 Hz, indole C(3)CH_2), 1.36 (d, 3H, J=7.0Hz, $HCCH_3$), 0.63 (m, 2H, CH_2Si), 0.0 (s, 9H, $Si(CH_3)_3$); Anal.Calcd. for $C_{17}H_{26}N_2O_3Si$ (Mw 334.494): C, 61.04; H, 7.83; N, 8.37. Found: C, 60.83; H, 7.93; N, 8.16.

Ethyl α-(4,4-dimethoxybutyloxamino)-β-(indol-3-yl)propanoate (26) via 19→22.

A stirring solution of **19** (1.57 g, 4 mmol), 4-bromo-1,1-dimethoxybutane[27] (1.58 g, 8 mmol) and K_2CO_3 (0.83 g, 6 mmol) in DMSO (25 mL) was kept at 45°C for 24 hours. The reaction mixture was diluted with CH_2Cl_2 (100 mL) and most of the DMSO was removed by washing with water and 0.1 N HCl. The resulting organic layer was washed with brine and dried with Na_2SO_4. Evaporation of the solvent gave a oil which was subjected to column chromatography ($CHCl_3$/n-hexane, 90/10, v/v) to give 1.37 g (67%) of **22.** Oil; Rf 0.58 (solvent system A); EIMS (70 eV) m/z (relative intensity) 508 (M^+, 4), 476 ([M-MeOH]$^+$, 5), 215 ([$C_{13}H_{13}NO_2$]$^+$, 95), 130 ([C_9H_8N]$^+$, 100); ^{1}H NMR δ 8.07 (br s, 1H, NH), 7.70-7.10 (m, 5H, indole C(2) and C(4)-C(7)H), 4.98 (X part of ABX spectrum, 1H, J=6.0 Hz, J=8.9 Hz, HCCOOEt), 4.37-3.74 (m, 7H, OCH_2CH_3, OCH_2CH_2Si, $NOCH_2$ and $HC(OMe)_2$), 3.50-3.30 (AB part of ABX spectrum, 2H, indole C(3)-CH_2), 3.30 (s, 6H, 2xOCH_3), 1.65 (m, 4H, $OCH_2CH_2CH_2CH$), 1.30 (t, 3H, OCH_2CH_3), 0.78 (m, 2H, CH_2Si), 0.0 (s, 9H, $Si(CH_3)_3$. A solution of **22** (1.35 g, 2.65 mmol) and tetrabutylammoniumfluoride (5.3 mL, 1N solution in THF) in THF (25 mL) was stirred for 2 hours. The reaction mixture was washed with saturated $NaHCO_3$ and brine and dried with $MgSO_4$. Evaporation of the solvent gave crude **26** which was subjected to column chromatography ($CHCl_3$) to yield 706 mg (78%) of **26.** Oil; Rf 0.33 (solvent system A); UV (MeOH) λmax 224, 274(sh), 281, 289 nm; CIMS (100 eV) m/z (relative intensity) 365 ([M+1]$^+$, 33), 332 ([M-MeOH]$^+$, 41), 301 ([$C_{17}H_{21}N_2O_3$]$^+$, 95), 216 ([$C_{13}H_{14}NO_2$]$^+$, 94), 130 ([C_9H_8N]$^+$, 100); ^{1}H NMR δ 8.06 (br s, 1H, NH), 7.67-7.07 (m, 5H, indole C(2) and C(4)-C(7)H), 5.88 (br s, 1H, N_bH), 4.35 (br t, 1H, $HC(OMe)_2$), 4.11 (q, 2H, OCH_2CH_3),4.00 (m, 1H, HCCOOEt), 3.69 (br t, 2H, $NOCH_2$), 3.28 (s, 6H, 2xOCH_3), 3.13-2.96 (m, 2H, indole C(3)CH_2), 1.60 (m, 4H, $OCH_2CH_2CH_2CH$), 1.46 (t, 3H, OCH_2CH_3).

3-[2-(4,4-Dimethoxybutyloxamino)ethyl]indole (27) via 20→23

Sodium hydride (41 mg, 1.7 mmol) was added to a cooled (-10°C) stirred solution of **20** (500 mg, 1.56 mmol) in dry DME (10 mL) in an argon atmosphere. The reaction mixture was allowed to warm to room temperature by which H_2-evolution occurred. The resulting clear solution was added dropwise to a stirred solution of 4-bromo-1,1-dimethoxybutane[27] (368 mg, 1.87 mmol) and NaI (260 mg, 1.75 mmol) in DME (10 mL). After stirring for 24 h. the reaction mixture was diluted with EtOAc (25 mL),

washed with 0.1 N HCl and brine and then dried with $MgSO_4$. Evaporation of the solvent gave crude **23**, which was added to a solution of Bu_4NF (2 eq.) in THF. After stirring the reaction mixture for 2 hours, the solution was washed with saturated $NaHCO_3$, brine and dried with $MgSO_4$. Evaporation of the solvent gave crude **27**, which was subjected to column chromatography ($CHCl_3$/MeOH, 99/1, v/v) to yield 306 mg (67%) of **27**. Oil; Rf 0.28 (solvent system A); UV (MeOH) λmax 224, 274(sh), 281, 289 nm; EIMS (70 eV) m/z (relative intensity) 292(M^+, 7), 260 ([M-MeOH]$^+$, 14), 229 ([$C_{14}H_{17}N_2O$]$^+$, 16), 216 ([$C_{14}H_{16}N_2O$]$^+$, 14), 130 ([C_9H_8N]$^+$, 100); ^{1}H NMR δ 8.08 (br s, 1H, indole NH), 7.70-7.07 (m, 5H, indole C(2) and C(4)-C(7)H), 5.35 (br s, 1H, NH), 4.44 (br t, 1H, $HC(OMe)_2$), 3.78 (br t, 2H, $NOCH_2$), 3.36 (s, 6H, 2xOCH_3), 3.36-2.93 (m, 4H, indole C(3)CH_2CH_2), 1.71 (m, 4H, $OCH_2CH_2CH_2CH_3$).

3-[2-(3-carbomethoxypropyloxamino)ethyl]indole (28) via 20→24

The same procedure was followed as described for **27**. **20** (640 mg, 2 mmol), methyl 4-bromobutanoate[28] (400 mg, 2.2 mmol), NaH (53 mg, 2.2 mmol) and NaI (300 mg, 2 mmol) gave crude **24**. Deprotection with Bu_4NF (2 eq.) in THF gave after column chromatography ($CHCl_3$/MeOH, 99/1, v/v) 406 mg (74%) of **28**. Oil; Rf 0.30 (solvent system A); UV (MeOH) λmax 224, 274(sh), 281, 289 nm; EIMS (70 eV) m/z (relative intensity) 276 (M^+, 7), 146 ([$C_6H_{12}NO_3$]$^+$, 26), 144 ([$C_{10}H_{10}N$]$^+$, 11), 130 ([C_9H_8N]$^+$, 100); ^{1}H NMR δ 8.04 (br s, 1H, indole NH), 7.64-7.05 (m, 5H, indole C(2) and C(4)-C(7)H), 5.56 (br s, 1H, NH), 3.73 (t, 2H, $NOCH_2$), 3.68 (s, 3H, OCH_3), 3.31-2.90 (m, 4H, indole C(3)CH_2CH_2), 2.44 (t, 2H, CH_2COOMe), 1.91 (m, 2H, $OCH_2CH_2CH_2$).

3-[2-(3-carbomethoxypropyloxamino)propyl]indole (29) via 21→25

The same procedure was followed as described for **27**. **21** (300 mg, 0.9 mmol), methyl 4-bromobutanoate[28] (180 mg, 1 mmol), NaH (24 mg, 1 mmol) and NaI (150 mg, 1 mmol) gave crude **25**. Deprotection with Bu_4NF (2 eq.) in THF gave after column chromatography ($CHCl_3$/MeOH, 99/1, v/v) 167 mg (64%) of **29**. Oil; Rf 0.33 (solvent system A); UV (MeOH) λmax 224, 274(sh), 281, 289 nm; EIMS (70 eV) m/z (relative intensity) 290 (M^+, 14), 223 (34), 160 (165), 131 (85), 130 ([C_9H_8N]$^+$, 100); ^{1}H NMR δ 8.01 (br s, 1H, indole NH), 7.67-7.02 (m, 5H, indole C(2) and C(4)-C(7)H), 3.73 (t, 2H, $NOCH_2$), 3.66 (s, 3H, OCH_3), 3.34 (m, 1H, $CHCH_3$), 2.93 and 2.78 (AB part of ABX spectrum, 2H, ^{2}J=14.0 Hz, J=7.0 Hz, J=5.8 Hz, indole C(3)CH_2), 2.41 (t, 2H, CH_2COOMe), 1.89 (m, 2H, $CH_2CH_2CH_2COOMe$), 1.56 (br s, 1H, NH), 1.13 (d, 3H, $CHCH_3$).

Ethyl α-[N-(2-(trimethylsilyl)ethyloxycarbonyl)amino]-β-(indol-3-yl)propenoate (30)

A solution of **19** (157 mg, 0.4 mmol), 4-bromo-1,1-dimethoxybutane[27] (158 mg, 0.8 mmol) and KOtBu (67 mg, 0.6 mmol) in DMSO (5 mL) was stirred for 24 h. at room temperature. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and most of the DMSO was removed by washing with water and 0.1 N HCl. The resulting organic layer was washed with brine and dried with Na_2SO_4. Evaporation of the solvent gave an oil which was subjected to column chromatography ($CHCl_3$) to give 145 mg (97%) of **30**. Rf 0.37 ($CHCl_3$/MeOH, 97/3, v/v); EIMS (70 eV) m/z (relative intensity) 374 (M^+, 18), 287 (9), 258 (8), 155 (15), 73 (100); ^{1}H NMR δ 8.73 (br s, 1H, indole NH), 7.89 (s, 1H, indole C(3)CH=C), 7.89-7.71 and 7.50-7.23 (2xm, 5H, indole C(2)H and C(4)-C(7)H), 6.09 (br s, 1H, HN*TEOC*), 4.35 (q, 2H, OCH_2CH_3), 4.24 (m, 2H, OCH_2CH_2Si), 1.38 (t, 3H, OCH_2CH_3), 1.00 (m, 2H, OCH_2CH_2Si), 0.00 (s, 9H, $Si(CH_3)_3$); Anal. Calcd. for $C_{19}H_{26}N_2O_4Si$ (Mw 374.516): C, 60.94; H, 7.00; N, 7.48. Found: C, 60.98: H, 7.01; N, 7.44.

Cyclization of dimethoxy acetals under acid conditions.

rel-(6R,12bR)-6-ethoxycarbonyl-1,2,3,6,7,12,12b-heptahydro-[1,2]oxazino[2',3':1,2]pyrido[3,4-b]indole (4a) and rel-(6R,12bS)-6-ethoxycarbonyl-1,2,3,6,7,12,12b-heptahydro-[1,2]oxazino[2',3':1,2]pyrido[3,4-b]indole (4b)

A solution of **26** (650 mg, 1.79 mmol), TFA (228 mg, 2 mmol) in dichloromethane (20 mL) was stirred for 4 hours. The reaction mixture was monitored by TLC (n-hexane/EtOAc, 75/25, v/v). The reaction mixture was washed with 0.1 N $NaHCO_3$ and brine and dried with Na_2SO_4. After evaporation of the solvent the crystalline material was subjected to column chromatography (n-hexane/EtOAc, 75/25, v/v) to yield 415 mg (77%) of **4a** and 110 mg (20%) of **4b**.

Compound 4a: Crystallized from EtOAc/n-hexane; mp 156-158°C; Rf 0.16 (solvent system C); UV (MeOH) λmax 225, 272(sh), 281, 289 nm; EIMS (70 eV) m/z (relative intensity) 300 (M^+, 22), 227 ([M-COOEt]$^+$, 100), 169 ([$C_{11}H_9N_2$]$^+$, 24), 130 ([C_9H_8N]$^+$, 48); ^{1}H NMR (400 MHz) δ 7.77 (br s, 1H,

NH), 7.42 (d, 1H, C(11)H), 7.32 (d, 1H, C(8)H), 7.18-7.08 (m, 2H, C(9) and C(10)H), 4.32 (m, 2H, OCH_2CH_3), 4.15-3.99 (m, 3H, C(12b)H, $NOCH_2$), 3.81 (X part of ABX spectrum, 1H, J=5.1 Hz, J=10.8 Hz, C(6)H), 3.18 and 3.10 (AB part of ABX spectrum, 2H, ^{2}J=15.1 Hz, J=5.1 Hz, J=10.8 Hz, C(7)H_2), 2.20 (m, 1H, C(1)H), 1.96 (m, 2H, C(1)H and C(2)H), 1.76 (m, 1H, C(2)H), 1.34 (t, 3H, OCH_2CH_3); Anal.Calc. for $C_{17}H_{20}N_2O_3$ (Mw 300.358): C, 67.98; H, 6.71; N, 9.33. Found: C, 68.21; H, 6.72; N, 9.25. The structure has been secured by single crystal X-ray crystallography[21].

Compound 4b: Crystallized from EtOAc/n-hexane; mp 145-147°C; Rf 0.21 (solvent system C); UV (MeOH) λmax 225, 272(sh), 281, 289 nm; CIMS (100 eV) m/z (relative intensity) 301 ([M+1]$^+$, 86), 300 (M$^+$, 52), 256 (34), 231 (75), 227 ([M-COOEt]$^+$, 100), 216(60); ^{1}H NMR (400 MHz) δ 7.77 (br s, 1H, NH), 7.44 (d, 1H, C(11)H), 7.30 (d, 1H, C(8)H), 7.16-7.07 (m, 2H, C(9) and C(10)H), 4.99 (d, 1H, J=10.4 Hz, C(12b)H), 4.35 (X part of ABX spectrum, 1H, J=1.25 Hz, J=7.45 Hz, C(6)H), 4.18-4.04 (m, 4H, OCH_2CH_3, $NOCH_2$), 3.36 (A part of ABX spectrum, 1H, ^{2}J=16.0 Hz, J=7.45 Hz, C(7)H_A), 3.09 (B part of ABX spectrum, 1H, ^{2}J=16.0 Hz, J=1.25 Hz, C(7)H_B), 2.21 (m, 1H, C(1)H), 1.91 (m, 1H, C(1)H), 1.74 (m, 2H, C(2)H_2), 1.23 (t, 3H, OCH_2CH_3); Anal.Calc. for $C_{17}H_{20}N_2O_3$ (Mw 300.358): C, 67.98; H, 6.71; N, 9.33. Found: C, 68.16; H, 6.70; N, 9.23.

1,2,3,6,7,12,12b-Heptahydro[1,2]oxazino[2',3':1,2]pyrido[3,4-b]indole (5)

The same procedure was followed as described for **4**. **27** (570 mg, 1.95 mmol), TFA (250 mg, 2.2 mmol) in dichloromethane (20 mL) gave 410 mg (92%) of **5**. mp 178-180 °C (EtOAc / n-hexane); Rf 0.49 (solvent system A); UV (MeOH) λmax 225, 273(sh), 281, 289 nm; EIMS (70 eV) m/z (relative intensity) 228 (M$^+$, 100), 197 (21), 169 ([$C_{11}H_9N_2$]$^+$, 81), 156 (34), 144 (26), 130 ([C_9H_8N]$^+$, 18); ^{1}H NMR (400 MHz) δ 7.77 (br s, 1H, NH), 7.46 (d, 1H, C(11)H), 7.31 (d, 1H, C(8)H), 7.17-7.08 (m, 2H, C(9) and C(10)H), 4.06 (br s, 2H, C(3)H_2), 3.86 (br s, 1H, C(12b)H), 3.57 (br s, 1H, C(6)H), 3.11-2.97 (m, 2H, C(6)H and C(7)H), 2.83 (d, 1H, C(7)H), 2.18 (d, 1H, C(1)H), 1.79 (m, 3H, C(1)H and C(2)H_2); Anal.Calc. for $C_{14}H_{16}N_2O$ (Mw 228.295) : C, 73.66; H, 7.06; N, 12.27. Found : C, 73.44, H, 7.70; N, 11.94.

Reductive ring closure

1,2,3,6,7,12,12b-Heptahydro[1,2]oxazino[2',3':1,2]pyrido [3,4-b]indole (5)

DIBAL (1N, 3 mL) in toluene was added dropwise to a cooled (-70°C) stirring solution of **28** (390 mg, 1.4 mmol) in dry toluene (75 mL) in an argon atmosphere. After stirring for 1.5 hours at -70°C, TFA (640 mg, 5.6 mmol) in dichloromethane (5 mL) was added carefully. Stirring was continued for 30 minutes at -70°C. After completion of the reaction as was monitored by TLC (solvent system A), the reaction mixture was poured into ice-water (100 mL). The organic layer was separated. The neutralized aqueous layer was extracted with EtOAc. The combined organic layers were washed with brine, dried with $MgSO_4$, filtered and the solvent evaporated to give crude **5**. Column chromatography ($CHCl_3$MeOH, 99/1, v/v) gave 241 mg (76%) of **5**. (For spectroscopic data, *vide supra*).

rel-(6R,12bR)-6-methyl-1,2,3,6,7,12,12b-heptahydro[1,2]oxazino[2',3 ':1,2]pyrido[3,4-b]indole (6a) and rel-(6R,12bS)-6-methyl-1,2,3,6,7,12,12b-heptahydro[1,2]oxazino[2',3' :1,2]pyrido-[3,4-b]indole (6b)

The same procedure was followed as described for **5**. From **29** (165 mg, 0.57 mmol) and DIBAL (2 eq.) was obtained a mixture of two diastereomers, which were separated by column chromatography ($CHCl_3$) to yield 93 mg (68%) of **6a** and 13 mg (9%) of **6b**.

Compound 6a: mp 194-196°C (EtOAc/n-hexane); Rf 0.34 (solvent system D); UV (MeOH) λmax 224, 273(sh), 281, 289 nm; EIMS (70 eV) m/z (relative intensity) 242 (M$^+$, 100), 227 ([M-CH_3]$^+$, 10), 200 (15), 183 ([$C_{12}H_{11}N_2$]$^+$, 53), 169 ([$C_{11}H_9N_2$]$^+$, 59), 168 ([$C_{11}H_8N_2$]$^+$, 45), 156 (28); ^{1}H NMR (400 MHz) δ 7.72 (br s, 1H, NH), 7.44 (d, 1H, J=7.6 Hz, C(11)H), 7.29 (d, 1H, J=7.9 Hz, C(8)H), 7.16-7.07 (m, 2H, C(9)-C(10)H), 4.12-4.00 (m, 2H, $NOCH_2$), 3.89 (d, 1H, J=9.7 Hz, C(12b)H), 3.14-3.06 (m, 1H, C(6)H), 2.88 and 2.67 (AB part of a ABX spectrum, 2H, ^{2}J=15.6 Hz, J=4.5 Hz, J=10.7 Hz, C(7)H_2), 2.14 (d, 1H, J=9.7 Hz, C(1)H), 1.87-1.71 (m, 3H, C(1)H and C(2)H_2), 1.39 (d, 3H, J=6.2 Hz, CH_3); ^{13}C NMR (400 MHz) δ 136.33 (C(11a)), 132.92 (C(12a)), 126.70 (C(7b)), 121.51 (C(10)), 119.53 (C(9)), 118.18 (C(8)), 110.82 (C(11)), 107.54 (C(7a)), 70.11 (C(3)), 62.35 (C(6)), 57.95 (C(12b)), 29.82 (C(1)), 28.10 (C((2)), 25.15 (C(7)), 19.46 (CH_3); Anal.Calc. for $C_{15}H_{18}N_2O$ (Mw 242.322): C, 74.35; H, 7.49; N, 11.56. Found: C, 74.34; H, 7.37; N, 11.47.

Compound 6b: mp 173-176°C (EtOAc/n-hexane); Rf 0.25 (solvent system D); UV (MeOH) λmax 224, 273(sh), 281, 289 nm; EIMS (70 eV) m/z (relative intensity) 242 (M$^+$, 100), 227 ([M-CH_3]$^+$, 14),

200 (18), 183 ($[C_{12}H_{11}N_2]^+$, 56), 169 ($[C_{11}H_9N_2]^+$, 72), 168 ($[C_{11}H_8N_2]^+$, 51), 156 (36); ^{1}H NMR (400 MHz) δ 7.76 (br s, 1H, NH), 7.46 (d, 1H, J=7.6 Hz, C(11)H), 7.32 (d, 1H, J=7.9 Hz, C(8)H), 7.17-7.08 (m, 2H, C(9)-C(10)H), 4.03 (br s, 2H, $NOCH_2$), 3.81 (m, 1H, C(12b)H), 3.24-3.18 (m, 1H, C(6)H), 2.57 (br d, 1H, C(7)H), 2.22-2.18 (m, 1H, C(7)H), 1.89-1.67 (m, 4H, $C(1)H_2$ and $C(2)H_2$), 1.16 (d, 3H, J=6.6 Hz, CH_3); Anal.Calc. for $C_{15}H_{18}N_2O$ (Mw 242.322): C, 74.35; H, 7.49; N, 11.56. Found: C, 74.16; H, 7.41; N, 11.36.

***Cis* 1-(3-Hydroxypropyl)-3-(ethoxycarbonyl)-1,2,3,4-tetrahydro-β-carb oline (34a)**

Activated zinc dust (100 mg) was added to a stirred solution of **4a** (90 mg, 0.3 mmol) in glacial acetic acid (20 mL). Subsequently the reaction mixture was kept at 80°C and monitored by TLC (solvent system B). The reaction was completed after 5 hours. The reaction mixture was filtered and the filtrate concentrated to dryness. The residue was dissolved in dichloromethane and this solution was washed successively with saturated $NaHCO_3$, water, brine and then dried with Na_2SO_4. The solvent was evaporated and the residue subjected to column chromatography ($CHCl_3$/ MeOH, 99/1, V/v) to yield 77 mg (85%) of **34a**. Rf 0.20 (solvent system B); CIMS (100 eV) m/z (relative intensity) 303 ($[M+1]^+$, 74), 302 (M^+, 41), 286 (25), 243 ($[C_{14}H_{15}N_2O_2]^+$, 100), 233 (40), 202 (37), 169 ($[C_{11}H_9N_2]^+$, 21); ^{1}H NMR δ 8.57 (br s, 1H, N(9)H), 7.52-7.05 (m, 4H, C(5)-C(8)H), 4.24 (q, 2H, OCH_2CH_3), 4.24-4.13 (m, 1H, C(1)H), 3.74 (X part of ABX spectrum, 1H, C(3)H), 3.60 (t, 2H, $CH_2CH_2CH_2OH$), 3.27 (s, 2H, N(2)H and OH), 3.08 and 2.78 (AB part of ABX spectrum, 2H, ^{2}J=15.8 Hz, J=12 Hz, J=4.1 Hz, $C(4)H_2$), 2.12-1.58 (m, 4H, $CH_2CH_2CH_2OH$), 1.33 (t, 3H, OCH_2CH_3); ^{13}C NMR (400 MHz) δ 172.85 (COOEt), 136.13 (C(8a)), 134.74 (C(9a)), 127.08 (C(4b)), 121.74 (C(7)), 119.49 (C(6)), 117.90 (C(5)), 110.91 (C(8)), 108.18 (C(4a)), 62.23 (CH_2OH), 61.26 (OCH_2CH_3), 56.38 (C(3)), 52.60 (C(1)), 32.26 ($C(1)CH_2$), 28.72 ($CH_2CH_2CH_2OH$), 25.62 (C(4)), 14.18 (OCH_2CH_3).

***Trans* 1-(3-Hydroxypropyl)-3-(ethoxycarbonyl)-1,2,3,4-tetrahydro-β-carboline (34b)**

The same procedure was followed as described for **34a**. From **4b** (90 mg. 0.3 mmol) was obtained 81 mg (89%) of **34b**. Rf 0.20 (solvent system B); CIMS (100 eV) m/z (relative intensity) 303 ($[M+1]^+$, 100), 302 (M^+, 89), 286 (21), 243 ($[C_{14}H_{15}N_2O_2]^+$, 79), 233 (42), 202 (38), 169 ($[C_{11}H_9N_2]^+$, 26); ^{1}H NMR δ 8.28 (br s, 1H, N(9)H), 7.54-7.04 (m, 4H, C(5)-C(8)H), 4.31-4.09 (m, 1H, C(1)H), 4.21 (q, 2H, OCH_2CH_3), 3.94 (X part of ABX spectrum, 1H, C(3)H), 3.68 (t, 2H, $CH_2CH_2CH_2OH$), 3.29 (s, 2H, N(2)H and OH), 3.12 and 2.92 (AB part of ABX spectrum, 2H, ^{2}J=15.0 Hz, J=5.4 Hz, J=7.6 Hz, $C(4)H_2$), 2.06-1.62 (m, 4H, $CH_2CH_2CH_2OH$), 1.28 (t, 3H, OCH_2CH_3); ^{13}C NMR (400 MHz) δ 173.35 (COOEt), 135.96 (C(8a)), 135.03 (C(9a)), 126.77 (C(4b)), 121.54 (C(7)), 119.15 (C(6)), 117.87 (C(5)), 110.84 (C(8)), 106.52 (C(4a)), 62.23 (CH_2OH), 61.13 (OCH_2CH_3), 51.90 (C(3)), 50.30 (C(1)), 33.16 ($C(1)CH_2$), 29.67 ($CH_2CH_2CH_2OH$), 24.93 (C(4)), 14.04 (OCH_2CH_3).

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12. Compound **18a** -the analogous N-alkoxamine derivative of **11**- has never been detected.
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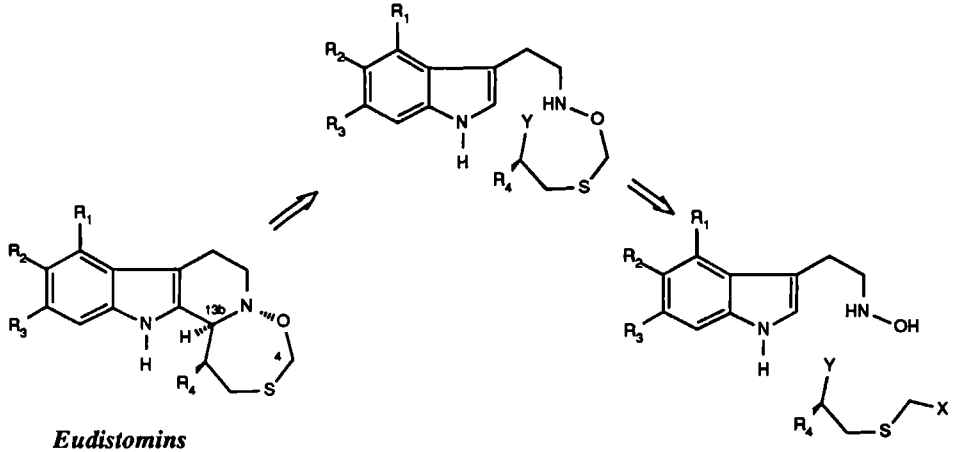
CHAPTER 7

The intramolecular Pictet-Spengler reaction of N-alkoxy-tryptamine derivatives in the stereoselective syntheses of Eudistomin derivatives.



Eudistomins

		R_1	R_2	R_3	R_4
L	a:	H	Br	H	NH_2
K	b:	H	H	Br	NH_2
C	c:	H	OH	Br	NH_2
E	d:	Br	OH	H	NH_2
	e:	H	H	H	NH_2
	f:	H	OMe	H	NH_2
	g:	H	H	H	H

"Intramolecular Pictet-Spengler reaction of N-alkoxytryptamines I. Synthesis of (±)-deamino-debromoeudistomin L.", Pedro H.H. Hermkens, Jan H v. Maarseveen, Chris G Kruse, Hans W. Scheeren, *Tetrahedron Lett.* (**1989**), *30*, 5009.

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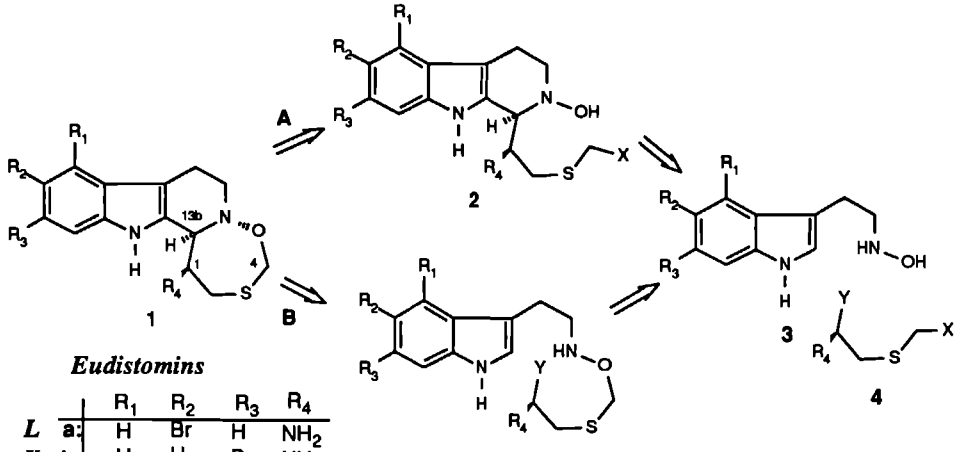
Chapter 7.1

INTRODUCTION

Recently, the intensive research on secondary metabolites with interesting pharmacological activities has led to the discovery of the class of indole alkaloids containing a tetrahydro-β-carboline fragment annulated with a oxathiazepine unit. These compounds *-the Eudistomins* **(1a-1d)** (Scheme I)- were isolated by Rinehart and Kobayashi from the colonial tunicate *Eudistoma olivaceum*[1]. More recently, Munro isolated the sulfoxide of eudistomin K (**1b**) and the unsubstituted eudistomin **1e** from *Ritterella sigillinoides*[2]. These compounds display potent antitumour activity and antiviral activity against *Herpes simplex* Type 1 (HSV-1) and *Polio* vaccine Type I viruses[1,2]. Because of its unique structure and its biological activity, this class of compounds constitutes a major challenge for total synthesis.

Scheme I



Eudistomins

		R_1	R_2	R_3	R_4
L	**a:**	H	Br	H	NH_2
K	**b:**	H	H	Br	NH_2
C	**c:**	H	OH	Br	NH_2
E	**d:**	Br	OH	H	NH_2
	e:	H	H	H	NH_2
	f:	H	OMe	H	NH_2
	g:	H	H	H	H

Since the eudistomins are accessible from natural sources in limited amounts, a total synthesis is desirable for allowing the preparation of structural analogues and for providing sufficient quantities of these compounds for extensive pharmacological studies.

Common in the approaches reported so far[3] is the construction of the C-ring of the β-carboline **2** as the first step by an *inter*molecular Pictet-Spengler reaction of the N-hydroxytryptamine **3** and a cysteinal derivative **4** (Route A, Scheme I). This Pictet-Spengler reaction occurred with high or complete stereoselectivity to give the stereoisomer having the desired *cis*-relationship between the C(1) and C(13b) protons. However, subsequent ringclosure **2**→**1** to form the oxathiazepine ring appears a difficult task and only recently two total syntheses of eudistomin L (**1a**) employing this approach have been reported with low overall yields.[4]

In chapter 6 we described[5] the synthesis of corynanthe analogs with an 1,2-oxazine as D-ring by an *intra*molecular Pictet-Spengler ringclosure of N-alkoxytryptamine derivatives. The feature of this approach is that ring C and D are formed simultaneously. We reasoned that by making use of the driving force of the Pictet-Spengler cyclization reaction this methodology could also lead to the construction of the unusual 7-membered oxathiazepine ring of eudistomin derivatives. In this chapter it is described that this approach is feasable indeed leading in high yields to the target molecules (Route B).

Accordingly the intramolecular Pictet-Spengler reaction of the N-alkoxytryptamine derivative **5** (Y=COOMe or $CH(OMe)_2$, R_1-R_4=H) -derived from **3** and **4** (X=CH_2Cl)- gave (±)-deamino-debromo-eudistomin L (**1g**) (Chapter 7.2).[6] Further application of this methodology is described in chapter 7.3 for the total synthesis of (-)-debromoeudistomin L (**1e**), of (-)-O-methyldebromoeudistomin E (**1f**) and their stereoisomers. The approach employed features the reaction of the N-alkoxytryptamine derivatives **5**, derived from the N-hydroxytryptamine **3** and a derivative of chloromethylcysteine (**4**, X=CH_2Cl, R_4=HNBOC, Y=COOMe).[7]

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5. See also, Hermkens, P.H.H.; Maarseveen, J.v.; Berens, H.C.; Smits, J.M.M.; Kruse, C.G.; Scheeren, J.W., *J.Org.Chem.*, in press.
6. Hermkens, P.H.H.; Maarseveen, J.v.; Kruse, C.G.; Scheeren, J.W., *Tetrahedron Lett.*, **1989**, *30*, 5009.
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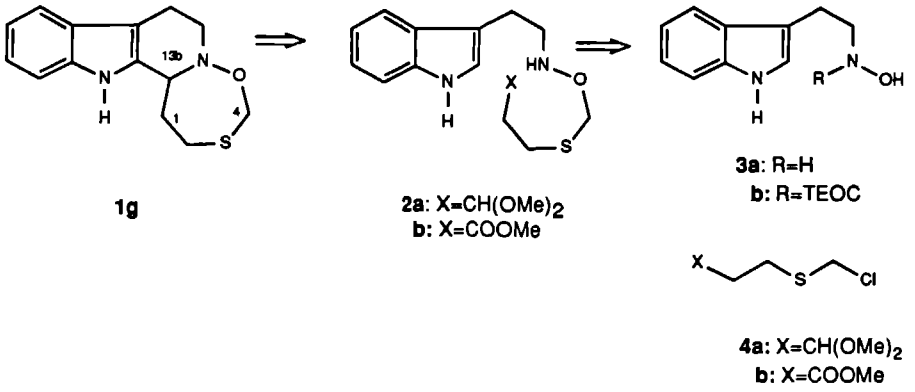
Chapter 7.2

SYNTHESIS OF DEAMINO-DEBROMOEUDISTIMIN L

Here we report the synthesis of (±)-deamino-debromoeudistomin L (**1g**) via an intramolecular Pictet-Spengler reaction of **2a** as well as **2b** (Scheme I)[1].

As the retrosynthetic scheme shows the intermediate for the intramolecular cyclization, *i.e.* **2**, should be constructed by a coupling reaction of **3a** and the corresponding chloromethylsulfides **4**. In chapters 2.1 and 6 it was described that TEOC-protected N-hydroxytryptamine (**3b**) can be O-alkylated by alkylhalogenides in the presence of NaI, followed by deprotection to give N-alkoxytryptamines in good yields in a one-pot synthesis. This methodology was employed here also for the synthesis of N-alkoxytryptamines **2**.

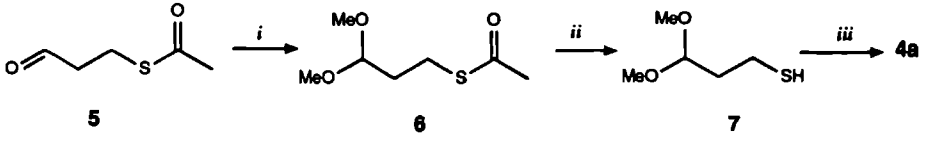
Scheme I



The chloromethyl sulfide **4a** could be prepared in 3 steps starting from the thioacetate **5**[2] (Scheme II). Acetalization of **5** with trimethyl orthoformate catalyzed by trifluoroacetic acid (TFA) (MeOH, 24h) occurred smoothly to give the dimethylacetal **6** (81% yield). Dropwise addition of a methanolic 1N NaOMe solution (1 equiv.) to **6** in methanol gave the thiol **7** (70% yield). A phase-transfer alkylation reaction of **7** with bromochloromethane using powdered potassium hydroxide (1.4 equiv.), catalyzed by triethylbenzylammoniumchloride (TEBAC; 0.1 equiv.) resulted in the quantitative formation of **4a**. The chloromethyl sulfide **4b** was prepared in an analogous fashion from methyl 3-mercaptopropionate (98% yield).

The freshly prepared O-anion of the N-hydroxyurethane derivative **3b** (see chapters 2.1 and 6) (DME/NaH(1.1 equiv.), 0°C) was added dropwise to the functionalized chloromethylsulfides **4a** or **4b** in the presence of NaI in DME at room temperature. The O-alkylated products were not isolated, but

Scheme II

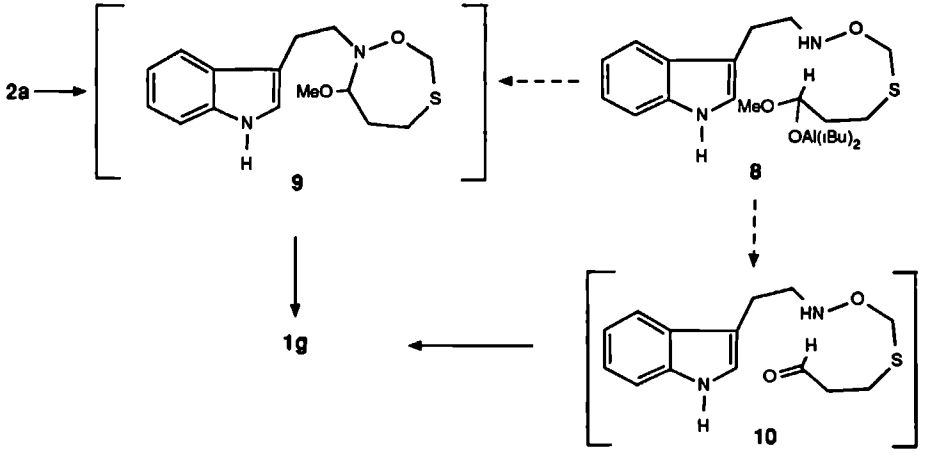


i) MeOH / HC(OMe)$_3$ / TFA ii) NaOMe iii) BrCH$_2$Cl / TEBAC / KOH

deprotected immediately by adding Bu_4NF (2 equiv.) to give the compounds **2a** and **2b** in an overall yield of 70% and 81%, respectively.

The desired intramolecular Pictet-Spengler reaction occurred on treatment of **2a** in dichloromethane with TFA (2 equiv.). After stirring for 3 days at room temperature, (±)-deamino-debromoeudistomin L (**1g**) was isolated in 71% yield. The NMR data of **1g** show a great resemblance with those of debromoeudistomin K (**1e**)[3], especially the data of the characteristic AB spectrum of the C(4)H protons of the oxathiazepine ring.

Subsequently we studied the formation of **1g** employing the methyl ester **2b** as precursor.[4] After reduction of **2b** with DIBAL (2 equiv.) at -70°C in toluene, TFA (3 equiv.) was added to the intermediate adduct **8** and intramolecular cylization occurred at -70°C within 15 minutes to give **1g** (61% yield). The rate of this extremely fast cyclization is much higher than that of the corresponding reaction employing **2a**, which can be rationalized either by the faster formation of the common aminal **9** or by the intermediacy of the corresponding aldehyde **10**.



In conclusion, it is demonstrated that the intramolecular Pictet-Spengler approach **2→1** is suitable for constructing the 7 membered oxathiazepine ring of the Eudistomin derivatives.

Spectroscopic data and Physical constants:

Compound 6: bp 74-75°C/4mm; η^{25} 1.4697; EIMS m/z 178 (M^+); ^{1}H NMR δ 4.45 (t, 1H, $HC(OMe)_2$), 3.34 (s, 6H, $2xOCH_3$), 2.92 (t, 2H, CH_2S), 2.35 (s, 3H, $COCH_3$), 1.84 (m, 2H, CH_2CH_2S).

Compound 7: bp 49°C/4mm; η^{25} 1.4571; EIMS m/z 136 (M^+); ^{1}H NMR δ 4.43 (t, 1, $HC(OMe)_2$), 3.27 (s, 6H, $2xOCH_3$), 2.47 (m, 2H, CH_2S), 1.92 (m, 2H, CH_2CH_2S), 1.40 (t, 1H, SH)

Compound 4a: bp 75-78 °C/3.5 mm; η^{25} 1.4970; ^{1}H NMR δ 4.73 (s, 2H, SCH_2Cl), 4.45 (t, 1H, $HC(OMe)_2$), 3.30 (s, 6H, $2xOCH_3$), 2.77 (t, 2H, CH_2S), 1.91 (m, 2H, CH_2CH_2S).

Compound 4b: CIMS m/z 184 ($[M+2]^+$), 182 (M^+), ^{1}H NMR δ 4.80 (s, 2H, SCH_2Cl), 3.73 (s, 3H, OCH_3), 3.18-2.58 (m, 4H, CH_2CH_2).

Compound 2a (Preparation procedure analogue to; chapter 6, compounds **26-27**)**:** Oil; Rf 0.56 ($CHCl_3$/MeOH, 97/3, v/v); CIMS m/z 325 ($[M+1]^+$), 292 ($[M-MeOH]^+$), 261 ($[C_{14}H_{17}N_2OS]^+$), 189 ($[C_{11}H_{13}N_2O]^+$), 130 ($[C_9H_8N]^+$); ^{1}H NMR δ 8.03 (br s, 1H, indole NH), 7.67-7.08 (m, 5H, indole C(2)H and C(4)-C(7)H), 5.89 (br s, 1H, NH), 4.87 (s, 2H, OCH_2S), 4.50 (t, 1H, $CH(OMe)_2$), 3.40 (s, 6H, $2xOCH_3$), 3.38 (m, 2H, CH_2N), 3.05 (m, 2H, indole C(3)CH_2), 2.72 (t, 2H, CH_2S), 1.99 (m, 2H, $CH_2CH(OMe)_2$).

Compound 2b (Preparation procedure analogue to; chapter 6, compounds **28-29**) **:**Oil; Rf 0.51 ($CHCl_3$/MeOH, 97/3, v/v); EIMS 308 (M^+), 188 ($[C_{11}H_{12}N_2O]^+$), 130 ($[C_9H_8N]^+$), 86 ($[C_4H_6O_2]^+$); ^{1}H NMR δ 8.03 (br s, 1H, indole NH), 7.62-7.00 (m, 5H, indole C(2)H and C(4)-C(7)H), 5.89 (br s, 1H, NH), 4.83 (s, 2H, OCH_2S), 3.64 (s, 3H, OCH_3), 3.07 (br t, 2H, CH_2N), 3.07-2.56 (m, 6H, indole C(3)CH_2 and SCH_2CH_2).

Compound 1g (Preparation procedure analogue to; chapter 6, compounds **4-6**)**:** mp 158-160°C (EtOAc/n-hexane); Rf 0.59 (n-hexane/EtOAc, 60/40, v/v); UV (MeOH) λmax 225, 274.5(sh), 281, 289.5 nm; CIMS (100 eV) m/z (relative intensity) 261 ($[M+1]^+$, 53), 260 (M^+, 100), 230 ($[C_{13}H_{14}N_2S]^+$, 34), 144 ($[C_{10}H_{10}N]^+$, 72), 130 ($[C_9H_8N]^+$, 86); ^{1}H NMR (400 MHz) δ 7.65 (br s, 1H, NH), 7.47 (d, 1H, ^{3}J=7.7 Hz, C(12)H), 7.31 (d, 1H, ^{3}J=7.7 Hz, C(9)H), 7.18-7.08 (m, 2H, C(10)-C(11)H), 5.08 and 5.01 (AB spectrum, 2H, ^{2}J=9.9 Hz, C(4)H_2), 4.12 (br s, 1H, C(13b)H), 3.67 (m, 1H, C(7)H), 3.11 (m, 1H, C(7)H), 3.09 (m, 1H, C(2)H), 3.05 (m, 1H, C(8)H), 2.82 (m, 1H, C(2)H), 2.78 (m, 1H, C(8)H), 2.65 (m, 1H, C(1)H), 2.10 (m, 1H, C(1)H); ^{13}C NMR (400 MHz) δ 136.38 C(12a), 133.07 C(13a), 126.59 C(8b), 121.75 C(11), 119.65 C(10), 118.31 C(9), 110.78 C(12), 108.13 C(8a), 73.44 C(4), 63.05 C(C13b), 54.06 C(7), 37.96 C(2), 26.49 C(8), 20.17 C(1); Anal.Calc. for $C_{14}H_{16}N_2OS$ (Mw 260.360): C, 64.59; H, 6.19; N, 10.76. Found: C, 64.25; H, 6.17; N, 10.68.

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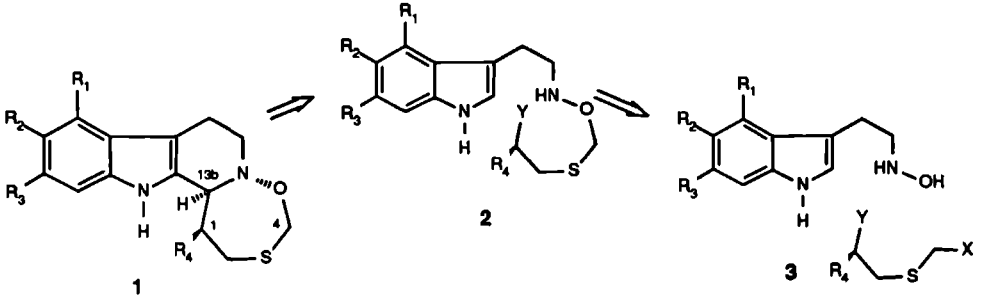
3. Nakagawa, M.; Liu, J.J.; Hino, T. *J.Am.Chem.Soc.* **1989**, *111*, 2721.
4. In chapter 6 we showed that the route starting with an ester at the end of the alkoxy chain (X=COOMe) underwent -after mild reduction and subsequent addition of TFA- the desired intramolecular Pictet-Spengler reaction much faster than the route employing the corresponding acetal derivatives (X=CH(OMe)$_2$).

Chapter 7.3

STEREOSELECTIVE SYNTHESIS OF (-)-DEBROMOEUDISTOMIN L, OF (-)-O-METHYL-DEBROMOEUDISTOMIN E AND OF THEIR STEREOISOMERS.

In chapter 7.2 we demonstrated[1] by synthesis of deamino-debromoeudistomin K (**1g**) that the intramolecular Pictet-Spengler reaction of N-alkoxytryptamine derivatives (*e.g.* **2**: Y=COOMe or $CH(OMe)_2$, R_1-R_4=H) -derived from **3** and **4** (X=CH_2Cl)- is a suitable approach of the 7-membered oxathiazepine ring occurring in the eudistomin derivatives (Scheme I).

Scheme I



	R_1	R_2	R_3	R_4
e:	H	H	H	NH_2
f:	H	OMe	H	NH_2
g:	H	H	H	H

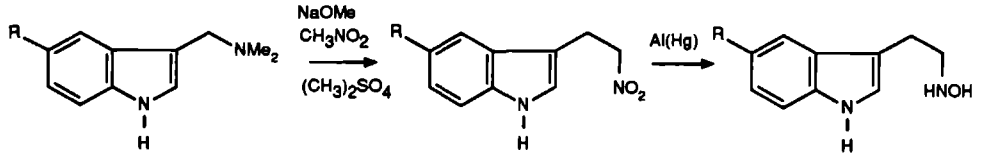
In this chapter we report the total synthesis of (-)-debromoeudistomin L (**1e**), of (-)-O-methyl-debromoeudistomin E (**1f**) and of their stereoisomers, by employment of this approach, *i.e.* the intramolecular Pictet-Spengler reaction of N-alkoxytryptamine derivatives (**2**), derived from N-hydroxytryptamine (**3**) and chloromethylcysteine derivative (**4**, X=CH_2Cl, R_4=HNBOC, Y=COOMe) (Route B, Scheme I).

Synthesis of the N-hydroxytryptamine derivatives.

Compound **9** was prepared via a known procedure[2], *viz.* nucleophilic displacement of the quaternarized amino function of gramine **5** with the anion of nitromethane to give **7** and subsequent reduction of the nitro group.(Scheme II).

5-Methoxy-N-hydroxytryptamine (**10**) was prepared in an analogous fashion. Reaction of 5-methoxygramine (**6**) with nitromethane in the presence of dimethyl sulfate and sodium methoxide

Scheme II



5: R=H
6: R=OCH_3

7: R=H
8: R=OCH_3

9: R=H
10: R=OCH_3

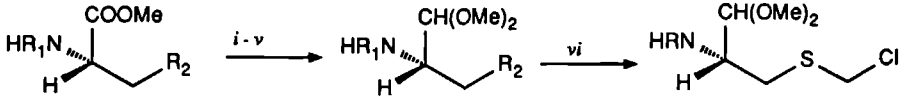
gave the nitro compound **8** (98%). Reduction of the nitro group by Al(Hg) in ethyl acetate saturated with water gave compound **10** (93%).

S-(chloromethyl)cysteine derivatives

From the synthesis of model compound **1g** we learned that compound **4** should contain the fragments X=CH_2Cl and Y=$CH(OMe)_2$ or COOMe. Thus, to obtain the correct stereochemistry at C(1) as present in the naturally occurring enantiomer of the target molecules a chloromethylsulfide derivative of D-cysteinal or D-cysteine methyl ester should be employed. Because of the tendency of cysteinal derivatives[3] to racemization and the high costs of D-cysteine derivatives, we decided to approach these compounds from D-serine methyl ester. In order to explore the feasibility of our approach we initially started with the more readily available L-serine methyl ester.

For the cysteinal derivatives (Y=$CH(OMe)_2$) we started with N-[(*tert*-butyloxy)carbonyl]-O-[(*tert*-

Scheme III



	R_1	R_2
11:	BOC	$OSiMe_2t$-Bu
12:	TrOC	$OSiMe_2t$-Bu

	R_1	R_2
13:	BOC	OH
14:		OTs
15:		SAc
16:		SH
17:	TrOC	OH
18:		OTs
19:		SAc
20:		SH

21: R=BOC
22: R=TrOC

i) DIBAL / toluene, -70°C ii) $HC(OMe)_3$ / MeOH / TFA iii) TsCl / pyridine iv) $CsCO_3$ / CH_3COSH / DMF v) NaOMe vi) $BrCH_2Cl$ / KOH / TEBAC

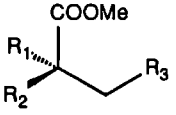
-butyl)dimethylsilyl]-L-serine methyl ester[4] (**11**) which was reduced with DIBAL in toluene at -70°C to the corresponding serinal derivative (Scheme III). The aldehyde was not isolated as such but immediately treated with trimethylorthoformiate in methanol and trifluoroacetic acid (TFA), whereby

the silyl protective group was removed and the dimethylacetal was formed in one-step giving **13** in 46% yield in an *e.e.* of 94% (*vide infra*).

The next step is the introduction of the sulphur atom. Recently, a method for the conversion of alcohols into thiols via reaction of the corresponding mesylate with cesium thiocarboxylates was published[5]. This method proved to be successful for our purpose also. Accordingly, the alcohol **13** was transformed into the corresponding tosylate **14**. Subsequent treatment with cesium thioacetate in DMF yielded the thioacetate **15** in 67% yield based on the alcohol **13**. This compound was converted quantitatively with sodium methoxide into the thiol **16**, which immediately was alkylated by a phase-transfer reaction with bromochloromethane using powdered KOH and triethylbenzylammonium chloride (TEBAC) to give the chloromethyl sulfide **21** in nearly quantitative yield[6]. Thus, the overall yield **11**→**21** is 31%.

In an analogous fashion *i.e.* **12**→**17**→**18**→**19**→**20**→**22**, the 2,2,2-(trichloro)ethyloxycarbonyl (TrOC) protected compound **22** was prepared in an overall yield of 14% (Scheme III). In contrast with their free aldehyde congeners[3], the dimethylacetal derivatives **15** and **19** did not undergo racemization during storage.

Chart I



	R_1	R_2	R_3
23a(L)	HNBOC	H	OTs
23b(D)	H	HNBOC	OTs
24a(L)	HNBOC	H	SAc
24b(D)	H	HNBOC	SAc
25a(L)	HNBOC	H	SCH_2Cl
25b(D)	H	HNBOC	SCH_2Cl

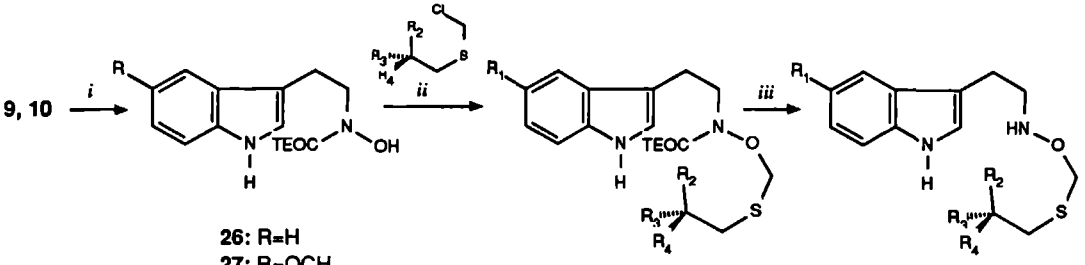
To obtain the cysteine methyl ester derivatives (Y=COOMe) the hydroxyl function of BOC-L-Ser-OMe and BOC-D-Ser-OMe was converted by the same procedure to the corresponding chloromethylsulfides (→**23a**→**24a**→**25a**; 71%) and (→**23b**→**24b**→**25b**; 77%) (Chart I). The sequential conversion of the OH into the SH group occurred without loss of enantiomeric purity.[8] We have no information on the optical purity of **25a** and **25b**.

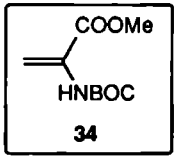
Coupling

We have demonstrated[9] that in a one-pot synthesis the 2-(trimethylsilyl)ethyloxycarbonyl (TEOC) protected N-hydroxytryptamine **26**[9] can be O-alkylated by alkylhalogenides, to give after N-deprotection N-alkoxytryptamines in good yields. This methodology was employed here also.

Accordingly, **10** was treated with 2(trimethylsilyl)ethyl chloroformate[10] in dichloromethane / dioxane to give **27** in 76% yield (Scheme IV). An efficient method for coupling of the chloromethylsulfides **21**, **22**, **25a** and **25b** with **26** was only found after several unsuccessful attempts (Scheme IV). Application of the basic-systems DMSO/KOtBu, DMSO/K_2CO_3, DME/KOtBu or benzene/$AgNO_3$/Et_3N[11] gave no reaction or unindentified products. The conditions of choice were analogous to those used in the preparation of methylthiomethyl ethers[12]. Compound **26** was treated

Scheme IV





	R_1	R_2	R_3	R_4	
28	H	$CH(OMe)_2$	HNBOC	H	**31**
29a	H	COOMe	HNBOC	H	**32a**
29b	H	COOMe	H	HNBOC	**32b**
30	OCH_3	COOMe	H	HNBOC	**33**

i) CH_2Cl_2 / dioxane, $Me_3SiCH_2CH_2OCOCl$ ii) NaH / DME, NaI iii) F⁻

with sodium hydride in dimethoxyethane (DME); the anion formed was subsequently coupled with the iodomethylsulfide formed *in situ* from **21** and sodium iodide in DME. The alkylated product **28** was not isolated, but N-deprotected immediately by adding tetrabutylammoniumfluoride (TBAF) to give the N-alkoxyamine **31** in an overall yield of 75%. The coupling failed however, when the TrOC-protected chloromethylsulfide **22** was used. Starting material **26** was recovered quantitatively. This failure is due to decomposition of **22** under the basic alkylation conditions used[13].

Following the same procedure employing now the methyl ester **25a** the dehydroamino acid derivative **34** (35%) (Scheme IV) and the desired thiomethyl ether **29a** (34%) were isolated together with recovered starting material **26** (59%). This β-elimination reaction could be prevented by adding the anion of **26** very slowly to the *in situ* prepared iodomethylsulfide of **25a**. The thiomethyl ether **29a** was now isolated in 85% yield. Deprotection with TBAF without isolation of **29a** gave **32a** in a moderate yield (48%). Likewise, deprotection of isolated **29a** with TBAF gave **32a** in only 61% yield. An alternative deprotection method[14] with a 'naked' fluoride generated by tetrabutylammonium chloride and potassium fluoride dihydrate in acetonitril at 60°C gave **32a** in high yield (85%). We do not have an explanation for the lower ability of TBAF to remove the TEOC-group of **29a** as compared to **28**.

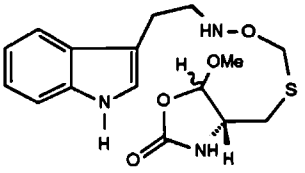
In a similar manner the D-cysteine derivative **25b** was coupled with **26** and **27** to give **29b** (84%) and **30** (88%), respectively. Deprotection of these compounds with the above mentioned fluoride reagent gave **32b** (86%) and **33** (81%), respectively.

The conversions **25a**→**29a** and **25b**→**29b/30** deserve further comment. Despite the application of alkaline conditions in the coupling-step we observed only minor losses of enantiomer purity in the end products (-)-**1e** and (-)-**1f** (*vide infra*). However, we found noticeable racemization when a slight excess of sodium hydride was used in the generation of the alkoxide anion.

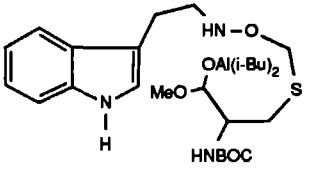
Cyclization

The intramolecular Pictet-Spengler reaction of the dimethylacetal **31** did not proceed as smoothly as the one we observed earlier[1,9] for N-alkoxytryptamine derivatives with a dimethylacetal function at the end of the alkoxy-chain. Treatment of **31** with TFA gave the two stereoisomers of the cyclic urethane **35** as the main products (Chart II). Apparently, the *in situ* formed alkoxy-carbenium ion is

Chart II



35



36

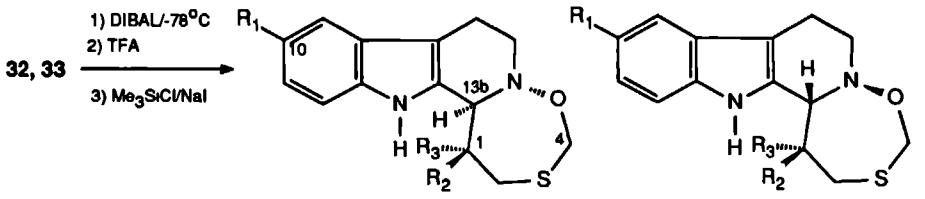
quenched intramolecularly by the BOC group[15]. Unfortunately, the cyclic urethane compounds decomposed on further treatment with acid.

We described earlier[1,9] that compound **4** (Y=COOMe, R_1-R_4=H) cyclized immediately after reduction of the methylester with DIBAL at -70°C and subsequent addition of TFA. The difference in reactivity of the intermediate obtained after the DIBAL reduction with that of the dimethylacetal derivatives was enormous, indicating a different mechanism in the Pictet-Spengler reaction to follow. Consequently, we reasoned that the undesired reaction we observed with the dimethylacetal **31** may be avoided by using the methylester derivatives **32** and **33**. Indeed, reduction of **32a** with DIBAL at -70°C in toluene followed by addition of TFA gave the cyclized products **37a** and **38a** in 52% yield (ratio **37a/38a**=71/29) (Scheme V and Table I, Entry 1). Presumably, cyclization under acidic conditions occurs either via the activated mixed-acetal **36** (Chart II) or via the corresponding free aldehyde.

The ^{1}H NMR data of compound **38a** show a great resemblance with those reported by Nakagawa[16] for the compound with a *cis* relationship between the protons of C(1) and C(13b). The characteristic differences between the spectra of **37a** and **38a** are *i)* the down-field shift of the indole NH proton δ 9.98 (**38a**: δ 8.52) propably participating in a H-bridge with the nitrogen of the HNBOC-group on C(1)[17] and *ii)* the AB spectrum of the OCH_2S protons at δ 5.26 (**38a**: δ 4.98) and δ 4.77 (**38a**: δ 4.83) with ^{2}J=11.4 Hz (**38a**: ^{2}J=9.1 Hz). Therefore, the relative stereochemistry at the C(1) and C(13b) centers was tentatively assigned as *trans* in **37a**[18] and *cis* in **38a**.

Although the intermolecular Pictet-Spengler reaction of **9** with cysteinals proceeds with high diastereoselectivity yielding predominantly the *cis*-isomer[19], the corresponding intramolecular cyclization shows a low diastereoselectivity towards the *trans*-isomer. The complexity of the cyclization mechanism[20] makes it difficult to give a rationale explanation for this reversal in the

Scheme V



	R_1	R_2	R_3	
37a	H	H	HNBOC	**38a**
38b	H	HNBOC	H	**37b**
39	OCH_3	HNBOC	H	**40**
41a	H	H	NH_2	**(+)-1e**
(-)-1e	H	NH_2	H	**41b**
(-)-11	OCH_3	NH_2	H	**42**

Table I. Cyclization of the N-Alkoxytryptamines **32** and **33**.

Entry	compound	solvent	cyclization condition	TFA (equiv.)	yield[a] (%)	cis/trans[b]
1	**32a**	toluene	-70°C/3h.	5	52	29/71
2		toluene	RT/1h.	5	41	39/61
3		DME	-70°C/3h.	5	70	13/87
4		CH_2Cl_2	-70°C/3h.	5	52	31/69
5		CH_2Cl_2	-90°C/0.5h.	15	58	41/59
6	**32b**	CH_2Cl_2	-90°C/0.5h.	15	53	34/66
7	**33**	CH_2Cl_2	-90°C/0 5h.	15	81	30/70

a) These yields are based on starting material and refer to isolated compounds b) Ratios are based on isolated compounds These are in agreement with the product ratios determined by HPLC

stereochemical outcome of the reactions.

We studied the cyclization by varying the solvent, the cyclization temperature and the amount of TFA used (Table 1, Entries 1-5). The ratio **37a**(*trans*)/**38a**(*cis*) depends slightly on the solvent used. Whereas for all the solvents used the major product has a *trans* relationship between the C(1) and C(13b) protons it was observed that toluene and dichloromethane give a higher ratio with respect to the *cis* compound than dimethoxyethane does (Entries 1, 3-4). Raising the temperature to room temperature gave a slight improvement of the ratio with respect to the *cis*-isomer, the yield dropped however (Entry 2). The best result was obtained when 15 equivalents TFA were added at once to a cooled solution (-90°C) of the reactant in dichloromethane (Entry 5). Consequently, the compounds **32b** and **33** were cyclized under these conditions to give **37b** and **38b** in 53% yield (ratio **37b/38b**=66/34) and **39** and **40** in 81% yield (ratio **39/40**=30/70), respectively (Scheme V, Table I, Entries 6-7). The stereochemistry of **37b**, **38b**, **39** and **40** was assigned by comparing their ^1H NMR

spectra with those of **37a** and **38a**.

The specific rotations of **38a** and **38b** are nearly identical to those reported by Nakagawa[19], who established the homochirality of these eudistomin derivatives by NMR techniques.

Deprotection

The most widely used procedure for removal of the BOC-group involves treatment with TFA at O°C[21]. However, we found that under these conditions **38b** gave the desired amine (-)-**1e** in low yield only due to decomposition of the latter. The method of choice appeared to be a mild deblocking method reported by Stammer[22a]: it consists of cleavage of the the carbamate by trimethylsilyl iodide (TMSI) at room temperature in acetonitrile. Compound **38b** was treated for 2 hours with the *in situ* generated TMSI (2 equivalents TMSCl and NaI)[22b] at room temperature in CH_3CN to give debromoeudistomin L ((-)-**1e**) in 94% yield (Scheme V). The deprotections **37a**→**41a**, **37b**→**41b**, **38a**→(+)-**1e**, **39**→(-)-**1f** and **40**→**42** proceeded in an analogous fashion in yields of 95%, 89%, 98%, 95% and 78%, respectively[23]. The structure of **42** is verified by a single crystal X-ray analysis[24] (Figure I). Deprotection of the *cis*-isomers hardly affected their ^1H NMR spectra, whereas in case of

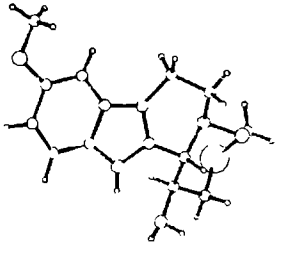


Figure I. PLUTO drawing of **42.**

the *trans*-isomers **41a**, **41b** and **42** the ^1H NMR spectra changed drastically. The differences that attract attention are peak-broadening, a single peak for OCH_2S at δ 4.94 and the up-field shift of C(13b)H. However, the ^{1}NMR spectrum of **41a** at -31°C showed peak-sharpening and clearly two conformations in a ratio of 82/18[25]. Significant differences between the conformers are the indole NH shifts (δ 10.34 *versus* 9.78) and the AB spectrum of OCH_2S (δ 4.99 and 4.97, ^2J=9.5Hz *versus* δ 5.37 and 4.88, ^2J=11.1Hz). The down-field shift of the indole NH proton of the *trans* compounds is probably a result of a H-bridge with the nitrogen of the amino-group on C(1).

Conclusion

In conclusion, we have shown that N-alkoxytryptamines with a methyl ester at the end of the alkoxy chain (**32a, 32b** and **33**) undergo -after reduction with DIBAL and subsequent treatment with TFA- an intramolecular Pictet-Spengler reaction to give eudistomin derivatives in reasonable yields. Cyclization occurs with a slight diastereoselective preference for the *trans*-isomer. Removal of the BOC group yields (-)-debromoeudistomin L (*i.e.* (-)-**1e**), its enantiomer (+)-**1e** and the two

diastereomers **41a** and **41b** or (-)-O-methyldebromoeudistomin E (*i.e.* (-)-**1f**) and its diastereomer **42**.

This facile approach gives access to all the four possible stereoisomers, and is applicable for the preparation of large amounts. Moreover it is sufficiently flexible allowing the prepration of analogues.

Experimental Section

Melting points were taken on a Koefler hot stage (Leitz-Wetzlar) and are uncorrected. Ultraviolet spectra were measured with a Perkin Elmer spectrometer, Model lambda 5. Proton magnetic resonance spectra were measured on a Bruker WH-90 or on a Bruker AM 400 spectrometer. Chemical shifts are reported as δ-values relative to tetramethylsilane as an internal standard; deuteriochloroform was used as solvent unless stated otherwise. Mass spectra were obtained with a double-focusing VG 7070E spectrometer. Thin-layer chromatography (TLC) was carried out by using silica gel F-254 plates (thickness 0.25 mm). Spots were visualized with a UV hand lamp, iodine vapor, or Cl_2-TDM.[27] For column chromatography Merck silica gel (type 60H) was used.

5-Methoxy-3-[2-nitroethyl]indole (8)

This synthesis is a modification of Cohen's procedure.[2] Sodium methoxide, which was freshly made from 1.22 g (53 mmol) sodium in dry methanol (50 mL) was added to a stirred solution of 5-methoxygramine (**6**) (9.8 g, 48 mmol), dimethylsulfate (12.1 g, 96 mmol) in nitromethane/methanol, 2/1, v/v (250 mL). After completion of the reaction (24 h) as was monitored by TLC ($CHCl_3$/MeOH, 99/1, v/v) most of the solvent was removed by evaporation in vacuo at room temperature. The residue was dissolved in dichloromethane and subsequently washed with 5% NH_4OH, 1N HCl and brine. The organic layer was dried (Na_2SO_4) and the solvent evaporated in vacuo. The residue was subjected to column chromatography ($CHCl_3$/MeOH, 99/1, v/v) to yield 10.34 g (98%) of **8**. ; EIMS (70 eV), m/z (relative intensity) 220 (M^+, 60), 174 ($[M\text{-}NO_2]^+$, 69), 173 ($[M\text{-}HNO_2]^+$, 100), 130 (57); ^1H NMR δ 8.00 (br s, 1H, NH), 7.30 (dd, 1H, J=8.7 Hz, C(7)H), 7.04-6.81 (m, 3H, C(2)H and C(4)H and C(6)H), 4.67 (t, 2H, CH_2NO_2), 3.89 (s, 3H, OCH_3), 3.47 (t, 2H, indole C(3)-CH_2).

5-Methoxy-3-[2-hydroxylamino-ethyl]indole (10)

To a stirred solution of **8** (9 g, 41 mmol) in EtOAc (saturated with water) freshly prepared Al(Hg) was added portionwise. After completion of the reaction (5 h) as was monitored by TLC ($CHCl_3$/MeOH, 93/7, v/v), the reaction mixture was filtered and the residue dried ($MgSO_4$) and the solvent evaporated in vacuo. The residue was crystallized from EtOAc/n-hexane to give 7.83 g (93%) of **10**. Rf 0.17 ($CHCl_3$/MeOH. 93/7, v/v); EIMS (70 eV) m/z (relative intensity) 206 (M^+, 21), 160 ($[C_{10}H_{10}NO]^+$, 100); ^1H NMR δ 7.94 (br s, 1H, NH), 7.25 (d, 1H, J=9.0 Hz, C(7)H), 7.04-6.74 (m, 3H, C(2),C(4) and C(6)H), 5.73 (br, s, 2H, HNOH), 3.84 (s, 3H, OCH_3), 3.34-2.90 (m, 4H, C(3)CH_2CH_2N); Anal.Calcd. for $C_{11}H_{14}N_2O_4$ (Mw 206.246): C, 64.06; H, 6.84; N, 13.58. Found: C, 64.30; H, 6.94; N, 13.36.

N-[(*tert*-Butyloxy)carbonyl]-O-[*tert*-butyldimethylsilyl]-L-Serine Methyl Ester (11)

To a stirred solution of L-BOC-Ser-OMe (20.9 g, 95.4 mmol) and imidazole (16.2 g, 240 mmol) in DMF (200 mL) was added dropwise tert-butyldimethylsilylchloride (15.9 g, 105 mmol). The reaction was stirred at 50°C for 5 days after which the solvent was removed in vacuo. The residue was dissolved in dichloromethane and successively washed with a 1N HCl solution and brine. The organic layer was dried (Na_2SO_4) and the solvent evaporated in vacuo to give crude **11**. The crude reaction product was subjected to column chromatography ($CHCl_3$) to give 28.15 g (89%) of **11**. Oil; Rf 0.61 ($CHCl_3$); $[\alpha]^{22}_D$ +7.5° (c=3.2, methanol); CIMS (100 eV) m/z (relative intensity) 334 ($[M+1]^+$,12), 278 (28), 260 ($[M\text{-}OC_4H_9]^+$, 13), 234 (49), 220 (43), 49 (100); ^1H NMR δ 5.32 (br d, 1H, NH), 4.33 (m, 1H, CHN), 3.95 and 3.76 (AB part ABX spectrum, 2H, ^{2}J=11.4 Hz, J=2.7 Hz, J=8.0 Hz, CH_2OSi), 3.71 (s, 3H, OCH_3), 1.43 (s, 9H, $C(CH_3)_3$), 0.85 (s, 9H, $SiC(CH_3)_3$), 0.00 (s, 6H, $Si(CH_3)_2$).

N-[2,2,2-(Trichloro)ethoxycarbonyl]-O-[*tert*-butyldimethylsilyl]-L-Serine Methyl Ester (12)

The same procedure was followed as described for **11**. L-TrOC-Ser-OMe (4.15 g, 14.1 mmol), imidazole (2.1 g, 31 mmol) and tert-butyldimethylsilylchloride (2.54 g, 16.9 mmol) in DMF (25 mL) gave after column chromatography ($CHCl_3$) 5.35 g (92%) of **12**: Rf 0.79 ($CHCl_3$/MeOH, 97/3, v/v); ^1H NMR δ 5.73 (br d, 1H, NH), 4.70 (s, 2H, CH_2CCl_3), 4.37 (m, 1H, $CHCH_2O$), 3.92 (m, 2H, $CHCH_2O$), 3.71 (s, 3H, OCH_3), 0.85 (s, 9H, $Si(C(CH_3)_3$), 0.00 (s, 6H, $Si(CH_3)_2$).

N-[(*tert*-Butyloxy)carbonyl]-L-Serinal Dimethylacetal (13)

To a stirred solution of **11** (28 g, 84 mmol) in dry toluene (250 mL) a solution of DIBAL in toluene (1.2 equiv.) was added in an argon atmosphere in such a rate that the temperature did not raise above -70°C. After stirring for 2 h. a 10% HCl/EtOH solution was added carefully. The reaction mixture was poured into 300 mL 10% aqueous HCl solution. The organic layer was separated and washed with brine. The organic layer was dried ($MgSO_4$) and the solvent evaporated in vacuo to give 25 g crude serinal derivative. Without further purification the crude reaction product (25 g, 83 mmol) was dissolved in methanol (250 mL) and trimethyl orthoformate (75 mL) and trifluoroacetic acid (1 mL) were added. After completion of the reaction (24 h) most of the solvent was removed by evaporation in vacuo. The residue was dissolved in dichloromethane and successively washed with an aqueous 1N $NaHCO_3$ solution and brine. The organic layer was dried (Na_2SO_4) and the solvent was evaporated in vacuo. The residue was subjected to column chromatography ($CHCl_3$/MeOH, 99/1, v/v) to give 9 g (46%) of **13** as a colourless oil. The yield is based on the ester **11**. Rf 0.31 ($CHCl_3$/MeOH, 97/3, v/v); $[\alpha]^{22}_D$-20[2] (c=3.4, methanol); CIMS (100 eV) m/z (relative intensity) 236 ($[M+1]^+$, 29), 204 ($[M-CH_3O]^+$, 11), 180 (20), 162 ($[M-C_4H_9O]^+$, 26), 148 (100); ^{1}H NMR δ 5.16 (br s, 1H, NH), 4.42 (d, 1H, $CH(OMe)_2$), 4.00-3.50 (m, 3H, $NCHCH_2$), 3.46 (s, 6H, 2xOCH_3), 2.61 (br s, 1H, OH), 1.46 (s, 9H, $C(CH_3)_3$); Anal.Calcd. for $C_{10}H_{21}NO_5$ (Mw 235.283): C, 51.05; H, 9.00; N, 5.95. Found: C, 50.99; H, 8.89; N, 5.88.

The enantiomeric excess of compound **13** was established by coupling it with (+)-α-methoxy-α-trifluoromethylphenylacetyl chloride according to the literature[32]. The obtained MTPA ester ($[\alpha]^{22}_D$-45.3° (c=2,65, methanol); ^{1}H NMR δ 7.51 (m, 2H, $C_6H_2H_3$), 7.40 (m, 3H, $C_6H_3H_2$), 4.74 (br d, 1H, NH), 4.42 (d, 2H, OCH_2), 4.25 (d, 1H, $CH(OMe)_2$), 4.10 (br s, 1H, OCH_2CH), 3.53 (q, 3H, CF_3COCH_3), 3.37 and 3.35 (2xs, 6H, 2x OCH_3), 1.42 (s, 9H, $C(CH_3)_3$).) showed in the NMR spectrum two signals for ^{19}F at 0.0912 ppm and -0.0012 ppm in a ratio of 3/97, respectively. Therefore the enantiomeric excess of compound **14** is 94%. A racemic mixture of **14** showed clearly two signals at 0.0896 ppm and -0.00103 ppm in ratio of 1/1.

N-[2,2,2-(Trichloro)ethoxycarbonyl]-L-Serinal Dimethylacetal (17)

The same procedure was followed as described for **13**. From **12** (2.85 g, 7 mmol), DIBAL (8.5 mmol) in toluene (25 mL) the serinal derivative was obtained which was converted without further purification with trimethyl orthoformate (7.5 mL), methanol (25 mL) and TFA (0.1 mL) to give after column chromatography ($CHCl_3$/MeOH, 99/1, v/v) 1.20 g (39%) of **17**; Rf 0.11 ($CHCl_3$/MeOH, 99/1, v/v); ^{1}H NMR δ 5.83 (br s, 1H, NH), 4.73 (s, 2H, CH_2CCl_3), 4.45 (d, 1H, $CH(OMe)_2$), 4.03-3.63 (m, 3H, $NCHCH_2$), 3.47 (s, 6H, 2xOCH_3), 2.92 (br s, 1H, OH).

General Procedure for the Preparation of the Thioacetates.

N-[(*tert*-Butyloxy)carbonyl]-S-[acetyl]-L-Cysteinal Dimethylacetal (15)

To a solution of the alcohol **13** (8.7 g, 37 mmol) in dry pyridine (60 mL) tosylchloride (7.75 g, 40.7 mmol) was added at -10°C. The reaction mixture was stirred at 4 °C overnight. After completion of the reaction as was monitored by TLC ($CHCl_3$/MeOH, 99/1, v/v), most of the pyridine was removed by evaporation in vacuo at room temperature. The residue was dissolved in dichloromethane and subsequently washed two times with a 2N $KHSO_4$ solution to remove the pyridine and then with water. The organic layer was dried (Na_2SO_4) and the solvent was evaporated in vacuo to give 13.3 g (93%) crude **14**.

To a suspension of $CsCO_3$ (7.06 g, 21.7 mmol) in dry DMF (85 mL) was added freshly distilled thioacetic acid (3.3 g, 43.3 mmol). As the $CsCO_3$ was dissolved, the crude tosylate **14** (13.3 g, 34.2 mmol) dissolved in 40 mL of dry DMF was added. The reaction mixture was stirred in the dark overnight and was kept under argon atmosphere. After completion of the reaction the solvent was evaporated in vacuo. The residue was dissolved in dichloromethane (200 mL) and washed with water. The organic layer was dried (Na_2SO_4) and concentrated in vacuo. The crude reaction product was subjected to column chromatography (EtOAc/n-hexane, 20/80, v/v) to give **15** (67%, based on the alcohol **13**): Rf 0.42 (EtOAc/n-hexane, 40/60, v/v); $[\alpha]^{22}_D$-70[4] (c=3.55, MeOH); CIMS (100 eV) m/z (relative intensity) 294 ($[M+1]^+$, 5), 262 ($[M-CH_3O]^+$, 12), 206 (76), 162 (21), 75 ($[C_2H_3OS]^+$, 100); ^{1}H NMR δ 4.78 (br d, 1H, NH), 4.27 (d, 1H, $CH(OMe)_2$), 4.07-3.76 (m, 1H, $CHCH_2S$), 3.44 (s, 6H, 2xOCH_3), 3.19 and 2.94 (AB part of ABX spectrum, 2H, ^{2}J=14.4 Hz, J=4.0 Hz, J=7.8 Hz, $CHCH_2S$), 2.34 (s, 3H, $SCOCH_3$), 1.43 (s, 9H, $C(CH_3)_3$).

N-[2,2,2-(Trichloro)ethoxycarbonyl]-S-[acetatyl]-L-Cysteinal Dimethylacetal (19)
The same procedure was followed as described for **15**. Compound **17** (585 mg, 1.88 mmol) gave via tosylate **18** after column chromatography (EtOAc/n-hexane, 80/20, v/v) 475 mg (69%) of **19**. The yield was based on the alcohol **15**; Rf 0.38 (EtOAc/n-hexane, 1/1, v/v); ^{1}H NMR δ 5.33 (br d, 1H, NH), 4.67 (s, 2H, CH_2CCl_3), 4.38 (d, 1H, $CH(OMe)_2$), 4.01 (m, 1H, $CHCH_2S$), 3.32 (s, 6H, 2xOCH_3), 3.11-2.93 (AB part of ABX spectrum, 2H, $CHCH_2S$), 2.35 (s, 3H, $SCOCH_3$).

N-[(*tert*-Butyloxy)carbonyl]-S-[acetatyl]-L-Cysteine Methyl Ester (25a)
The same procedure was followed as described for **15**. (L)-BOC-Ser-OMe (3.8 g, 17.4 mmol) gave via tosylate **23a** after column chromatography (EtOAc/n-hexane, 25/75, v/v) 3.85 g (80%) of **24a**: mp 46-47°C (EtOAc/n-hexane); Rf 0.51 (EtOAc/n-hexane, 50/50, v/v); $[\alpha]^{22}_D$-44[2] (c=2.6, MeOH); IR (KBr) ν(cm^{-1}) 3360 (NH), 1730 (C=O), 1680 (C=O); CIMS (100 eV) m/z (relative intensity) 278 ([M+1]$^+$, 6), 222 (66), 178 (100), 162 (96); ^{1}H NMR δ 5.23 (br d, 1H, NH), 4.61-4.38 (m, 1H, CHCOOMe), 3.74 (s, 3H, OCH_3), 3.52-3.16 (AB part of ABX spectrum, 2H, CH_2S), 2.33 (s, 3H, $SCOCH_3$), 1.42 (s, 9H, $C(CH_3)_3$); Anal.Calcd. for $C_{11}H_{19}NO_5S$ (Mw 277.340): C, 47.64; H, 6.90; N, 5.50. Found: C, 47.81; H, 6.89; N, 5.02.

N-[(*tert*-Butyloxy)carbonyl]-S-[acetatyl]-D-Cysteine Methyl Ester (24b)
The same procedure was followed as described for **15**. (D)-BOC-Ser-OMe (7.7 g, 35.2 mmol) gave via tosylate **23b** after column chromatography (EtOAc/n-hexane, 25/75, v/v) 8.2 g (84%) of **24b**: $[\alpha]^{22}_D$+45[9] (c=2.7, MeOH). Further spectroscopic data and mp are identical with **24a**.

General Procedure for the Preparation of the Chloromethylsulfides.

N-[(*tert*-Butyloxy)carbonyl]-S-[chloromethyl]-L-Cysteine Dimethylacetal (21)
To a stirred solution of thioacetate **15** (1.0 g, 3.4 mmol) in ethanol (15 mL) was added dropwise a 1N NaOEt solution in ethanol (3.5mL). After completion of the reaction, as was monitored by TLC (EtOAc/n-hexane, 1/2, v/v) the ethanol was removed by evaporation in vacuo. The residue was dissolved in dichloromethane and subsequently washed with 0.1N HCl and brine. The organic layer was dried (Na_2SO_4) and the solvent was evaporated in vacuo to give 860 mg (100%) crude **16**. The thiol was not further purified but dissolved in bromochloromethane (30 mL) and triethylbenzylammonium chloride (TEBAC) (77 mg, 0.34 mmol) and powdered KOH (267 mg, 4.76 mmol) were added successively. After 15 min. the reaction was complete. The reaction mixture was filtered and the filtrate subsequently washed with water, 1N HCl, and brine. The organic layer was dried (Na_2SO_4) and the solvent evaporated in vacuo to give 1.0 g (98%) of the chloromethylsulfide **21** which was not further purified. Oil; Rf 0.54 (EtOAc/n-hexane, 1/2, v/v); ^{1}H NMR δ 4.93 (br d, 1H, NH), 4.78 (s, 2H, SCH_2Cl), 4.27 (d, 1H, $HC(OMe)_2$), 4.13-3.70 (m, 1H, $CHCH_2S$), 3.45 (s, 6H, 2xOCH_3), 3.23-2.55 (AB part of ABX spectrum, 2H, $CHCH_2S$), 1.47 (s, 9H, $C(CH_3)_3$).

N-[2,2,2-(Trichloro)ethoxycarbonyl]-S-[chloromethyl]-L-Cysteine Dimethylacetal (22)
The same procedure was followed as described for **21**. Compound **19** (475 mg, 1.29 mmol) and NaOMe (1.35 mL 1N methanolic solution) gave via the thiol **20**, 244 mg (50%) of **22**: Rf 0.44 (EtOAc/n-hexane, 1/1, v/v); ^{1}H NMR δ 5.44 (br d, 1H, NH), 4.78 (s, 2H, SCH_2Cl), 4.69 (s, 2H, CH_2CCl_3),4.37 (d, 1H, $HC(OMe)_2$), 4.15-3.77 (m, 1H, $CHCH_2S$), 3.41 (s, 6H, 2xOCH_3), 3.18-2.67 (AB part of ABX spectrum, 2H, $CHCH_2S$).

N-[(*tert*-Butyloxy)carbonyl]-S-[chloromethyl]-L-Cysteine Methyl Ester (25a)
The same procedure was followed as described for **21**. Compound **24a** (6.5 g, 23 mmol) gave via the thiol derivative, 5.9 g (89%) of the chloromethylsulfide **25a**. oil; Rf 0.52 (EtOAc/n-hexane, 1/1, v/v); CIMS (100 eV) m/z (relative intensity) 286 ([M+3]$^+$, 3.65), 284 ([M+1]$^+$, 9.72), 230 (17), 228 (40), 192 (28), 186 (22), 184 (49), 148 (100); ^{1}H NMR δ 5.30 (br d, 1H, NH), 4.71 (s, 2H, SCH_2Cl), 4.70-4.51 (m, 1H, CHCOOMe), 3.78 (s, 3H, OCH_3), 3.29 and 3.15 (AB part of ABX spectrum, 2H, ^{2}J=14.0 Hz, J=4.9 Hz, J=5.7 Hz, SCH_2CH), 1.42 (s, 9H, $C(CH_3)_3$).

N-[(*tert*-Butyloxy)carbonyl]-S-[chloromethyl]-D-Cysteine Methyl Ester (25b)
The same procedure was followed as described for **21**. Compound **24b** (6.5 g, 23 mmol) gave via the thiol derivative, 6.0 g (92%) of the chloromethylsulfide **25b**. Identical spectroscopic data as for **25a**.

5-Methoxy-3-[2-(N,N-2-(trimethylsilyl)ethoxycarbonyl, hydroxy)aminoethyl]indole (27)

2-(Trimethylsilyl)ethylchloroformate[10] (8.12 g, 45 mmol) was added at room temperature dropwise to a stirred solution of **10** (6.2 g, 30 mmol) in CH_2Cl_2/dioxane (150 mL). After completion of the reaction (2 h), as was monitored by TLC ($CHCl_3$/MeOH, 93/7, v/v), most of the solvent was removed by evaporation in vacuo. The residue was dissolved in dichloromethane and subsequently washed with saturated $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and evaporated in vacuo. The residue was subjected to column chromatography (EtOAc/n-hexane, 40/60, v/v) to give 7.94 g (76%) of **27**. Rf 0.41 ($CHCl_3$/MeOH, 93/7, v/v); EIMS (70 eV) m/z (relative intensity) 350 (M^+, 15), 233 ($[M-C_5H_{13}OSi]^+$, 23), 218 (27), 174 ($[C_{11}H_{12}NO]^+$, 26, 173 (60), 160 ($[C_{10}H_{10}NO]^+$, 100); ^1H NMR δ 7.96 (br s, 1H, NH), 7.26 (d, 1H, ^3J=9.0 Hz, C(7)H), 7.11-6.80 (m, 3H, C(2), C(4) and C(6)H), 6.71 (br s, 1H, OH), 4.14-3.80 (m, 4H, CH_2N and CH_2O), 3.88 (s, 3H, OCH_3), 3.12 (t, 2H, C(3)-CH_2), 0.91-0.72 (m, 2H, CH_2Si), 0.00 (s, 9H, $Si(CH_3)_3$).

General Procedure for the Coupling of TEOC-protected N-hydroxytryptamines with Chloromethylsulfides.

Compound 31

Sodium hydride was added to a cooled (-10°C) stirred solution of **26** (960 mg), 3 mmol) in dry DME (15 mL) in an argon atmosphere. The reaction mixture was allowed to warm to room temperature (H_2-evolution occurred). To the resulting clear solution NaI (450 mg, 3 mmol) was added at once and then a solution of **21** (1123 mg, 3.75 mmol) in DME (15 mL). After completion of the reaction (5 h), as was monitored by TLC (EtOAc/n-hexane, 1/1, v/v) the alkylated product **28** was not isolated but Bu_4NF (9 mL, 1N solution in THF) was added. The reaction mixture was stirred overnight, after which it was diluted with EtOAc (50 mL) and subsequently washed with saturated $NaHCO_3$, 1N HCl, and brine. The organic layer was dried ($MgSO_4$) and the solvent evaporated in vacuo. The crude reaction product was subjected to column chromatography (EtOAc/n-hexane, 30/70, v/v) to give 988 mg (75%) of **31**. Oil; Rf 0.44 (EtOAc/n-hexane, 1/1, v/v); $[\alpha]^{22}_D$-23[6] (c=2.75, methanol); CIMS (100 eV) m/z (relative intensity) 440 ($[M+1]^+$, 60), 352 ($[C_{16}H_{21}N_3O_4S+1]^+$, 67), 189 ($[C_{11}H_{13}N_2O]^+$, 37), 176 (100), 144 ($[C_{10}H_{10}N]^+$, 61), 130 ($[C_9H_8N]^+$, 70); ^1H NMR δ 8.16 (br s, 1H, NH), 7.67-7.02 (m, 5H, indole C(2)H and C(4)-C(7)H), 5.98 (br s, 1H, HNO), 5.05 (br d, 1H, NHBOC), 4.91 (s, 2H, OCH_2S), 4.42 (d, 1H, $CH(OMe)_2$), 4.09-3.85 (m, 1H, $CHCH_2S$), 3.40 (s, 6H, 2x OCH_3), 3.40-2.64 (m, 6H, indole C(3)CH_2CH_2N and $CHCH_2S$), 1.48 (s, 9H, $C(CH_3)_3$).

Compound 29a

Procedure A

The same procedure was followed as described for **31**. Compound **26** (1g, 3.13 mmol), NaH (82 mg, 3.34 mmol), NaI (470 mg, 3.13 mmol) and **25a** (1g, 3.5 mmol) gave after column chromatography (EtOAc/n-hexane, 35/65, v/v) 248 mg (35%) **34**, 610 mg (34%) of **29a**, and 593 mg (59%) of starting material **26**.

Compound 34: CIMS (100 eV) m/z (relative intensity) 202 ($[M+1]^+$, 70), 146 (100), 102 ($[M-BOC+1]^+$, 79); ^1H NMR δ 7.02 (br s, 1H, NH), 6.17 (s, 1H, C=CH), 5.77 (d, 1H, J=1.5Hz, C=CH), 3.83 (s, 3H, OCH_3), 1.47 (s, 9H, $C(CH_3)_3$).

Compound 29a: Oil; Rf 0.34 (EtOAc/n-hexane, 1/2, v/v); $[\alpha]^{22}_D$-17[6] (c=2.1, methanol); CIMS (100 eV) m/z (relative intensity) 568 ($[M+1]^+$, 1), 567 (M^+, 2), 468 (9), 440 (15), 144 ($[C_{10}H_{10}N]^+$, 68), 130 ($[C_9H_8N]^+$, 48), 57 ($[C_4H_9]^+$, 100); ^1H NMR δ 8.04 (br s, 1H, NH), 7.68-7.02 (m, 5H, indole C(2)H and C(4)-C(7)H), 5.60 (br d, 1H, NHBOC), 4.89 (s, 2H, OCH_2S), 4.69-4.38 (m, 1H, CHCOOMe), 4.12-3.72 (m, 4H, CH_2N and $SiCH_2CH_2O$), 3.76 (s, 3H, OCH_3), 3.26-2.90 (m, 4H, indole C(3)-CH_2 and $CHCH_2S$), 1.41 (s, 9H, $C(CH_3)_3$), 1.00-0.68 (m, 2H, CH_2Si), 0.00 (s, 9H, $Si(CH_3)_3$).

Procedure B:

Sodium hydride (356 mg, 14.8 mmol) was added to a cooled (-10°C) stirred solution of **26**[9] (5 g, 15.6 mmol) in dry DME (75 mL) in an argon atmosphere. The reaction mixture was allowed to warm to room temperature (H_2-evolution occurred). The resulting clear solution was added dropwise to a stirred solution of **25a** (5.9 g, 20.8 mmol) and NaI (5 g, 33.3 mmol) in DME (75 mL). After stirring for 24 hours, the reaction mixture was concentrated in vacuo. The residue was dissolved in EtOAc and subsequently washed with 0.1N HCl and brine. The organic layer was dried ($MgSO_4$) and evaporated in vacuo. The residue was subjected to column chromatography ($CHCl_3$) to give 7.5 g (85%) of **29a**. Spectroscopic data *vide supra*.

Compound 29b
Following procedure (B) as described for **29a** with **26** (5 g, 15.6 mmol), NaH (356 mg, 14.8 mmol), chloromethylsulfide **25b** (5.9 g, 20.8 mmol) and NaI (5 g, 33.3 mmol) in DME (150 mL) gave after column chromatography ($CHCl_3$) 7.4 g (84%) of **29b**. $[\alpha]^{22}_D$+21[4] (c=3.7, methanol); Further spectroscopic data are identical with **30a**.

Compound 30
Following procedure (B) as described for **29a** with **27** (1.6 g, 4.6 mmol), NaH (105 mg, 4.37 mmol) and the chloromethylsulfide **25b** (2 g, 6.9 mmol) and NaI (1.5 g, 10 mmol) in DME (30 mL) gave after column chromatography ($CHCl_3$) 2.43 g (88%) of **30**. Oil; Rf 0.52 ($CHCl_3$/MeOH, 97/3, v/v); $[\alpha]^{22}_D$+16[7] (c=3.6, methanol); CIMS (100 eV) m/z (relative intensity) 598 $(M+1]^+$, 0.23), 597 (M^+, 0.22), 543 (0.33), 499 (0.75), 470 (1.3), 57 ($[C_4H_9]^+$, 100); ^{1}H NMR δ 7.93 (br s, 1H, NH), 7.23 (d, 1H, J=9.0 Hz, C(7)H), 7.03-6.74 (m, 3H, C(2), C(4) and C(6)H), 5.61 (br d, 1H, HNBOC), 4.88 (s, 2H, OCH_2S), 4.67-4.42 (m, 1H, CHCOOMe), 4.14-3.59 (m, 4H, CH_2N and $SiCH_2CH_2O$), 3.87 (s, 3H, indole C(5)-OCH_3), 3.78 (s, 3H, $COOCH_3$), 3.18-2.97 (m, 4H, indole C(3)-CH_2 and $CHCH_2S$), 1.40 (s, 9H, $C(CH_3)_3$), 1.00-0.64 (m, 2H, $SiCH_2$), 0.00 (s, 9H, $Si(CH_3)_3$).

General Procedure for the Removal of the TEOC-group.

Compound 32a
A solution of **29a** (6.2 g, 10.9 mmol), Bu_4NCl (9 g, 33 mmol) and $KF.2H_2O$ (4.1 g, 44 mmol) in dry acetonitrile (200 mL) was stirred for 10 h. at 55°C. The solvent was evaporated in vacuo. The residue was dissolved in EtOAc and successively washed with water, saturated NH_4Cl, and brine. The organic layer was dried ($MgSO_4$) and the solvent evaporated in vacuo. The crude reaction product was subjected to column chromatography ($CHCl_3$) to give 3.94 g (85%) of **32a**. Oil; Rf 0.38 (EtOAc/n-hexane, 1/1, v/v); $[\alpha]^{22}_D$-8[6] (c=1.85, methanol), CIMS 100 eV) m/z (relative intensity) 424 $([M+1]^+$, 27), 368 (34), 338 (8), 324 (15), 189 ($[C_{11}H_{13}N_2O]^+$, 47), 144 ($[C_{10}H_{10}N]^+$, 100), 130 ($[C_9H_8N]^+$, 72), 57 ($[C_4H_9]^+$, 89); ^{1}H NMR δ 8.06 (br s, 1H, NH), 7.63-7.00 (m, 5H, indole C(2)H and C(4)-C(7)H), 6.08 (br s, 1H, HNO), 5.91 (br d, 1H, NHBOC), 4.82 (s, 2H, OCH_2S), 4.71-4.51 (m, 1H, CHCOOMe), 3.71 (s, 3H, OCH_3), 3.37-2.82 (m, 6H, indole C(3)-CH_2CH_2 and $CHCH_2S$), 1.41 (s, 9H, $C(CH_3)_3$).

Compound 32b
Following the same procedure as described for **32a** with **29b** (6.2 g, 10.9 mmol), Bu_4NCl (9 g, 33 mmol) and $KF.2H_2O$ (4.1 g, 44 mmol) in dry acetonitrile (200 mL) gave after column chromatography ($CHCl_3$) 4.00 g (86%) of **32b**: $[\alpha]^{22}_D$+9[1] (c=3.2, methanol); Further spectroscopic data are identical with **32a**.

Compound 33
Following the same procedure as described for **32a** with **30** (2.25 g, 3.75 mmol), Bu_4NCl (3.07 g, 11.25 mmol) and $KF.2H_2O$ (1.4 g, 15 mmol) gave after column chromatography ($CHCl_3$/MeOH, 99/1, v/v) 1.38 g (81%) of **33**. Oil; Rf 0.42 ($CHCl_3$/MeOH, 97/3, v/v); $[\alpha]^{22}_D$+5[8] (c=4.5, methanol); CIMS 100 eV) m/z (relative intensity) 454 $([M+1]^+$, 9), 453 (M^+, 3), 398 (18), 354 (12), 219 (28), 191 (33), 174 ($[C_{11}H_{12}NO]^+$, 100), 160 ($[C_{10}H_{10}NO]^+$, 54); ^{1}H NMR δ 8.02 (br s, 1H, NH), 7.26 (d, 1H, C(7)H) 7.09-6.80 (m, 3H, C(2), C(4) and C(6)H), 6.11 (br s, 1H, HNO), 5.94 (br d, 1H, NHBOC), 4.90 and 4.85 (AB spectrum, 2H, ^{2}J=11.9 Hz, OCH_2S), 4.68-4.51 (m, 1H, CHCOOMe), 3.86 (s, 3H, OCH_3), 3.71 (s, 3H, $COOCH_3$), 3.41-2.90 (m, 6H, indole C(3)-CH_2CH_2 and $CHCH_2S$), 1.44 (s, 9H, $C(CH_3)_3$).

Cyclization Attempt of 31
Compound 35
A solution of **31** (110 mg, 0.25 mmol), TFA (114 mg, 1 mmol) in dichloromethane (12 mL) was stirred for 2 days. The reaction mixture was washed with 0.1 N $NaHCO_3$ and brine. The organic layer was dried (Na_2SO_4) and evaporated in vacuo. The residue was subjected to column chromatography ($CHCl_3$) to give unidentified products, 28 mg (25%) starting material (**31**) and 21 mg (24%) diastereomer a and 21 mg (24%) diastereomer b of **35**.
diastereomer a: CIMS (100 eV) m/z (relative intensity) 352 $([M+1]^+$, 23), 320 (56), 189 ($[C_{11}H_{13}N_2O]^+$, 39), 176 (100), 144 ($[C_{10}H_{10}N]^+$, 65), 130 ($[C_9H_8N]^+$, 88); ^{1}H NMR δ 8.27 (br s, 1H,

indole NH), 7.67-7.03 (m, 5H, C(2), C(4)-C(7)H), 6.46 (br s, 1H, NH), 6.21 (br s, 1H, HNO), 5.37 (d, 1H, J=6Hz, OCH(OMe)), 4.90 and 4.81 (AB spectrum, 2H, ^{2}J=12Hz, OCH_2S), 3.92 (m, 1H, $NCHCH_2$), 3.49 (s, 3H, OCH_3), 3.31 (t, 2H, CH_2NO), 3.08-2.68 (m, 4H, $NCHCH_2$, indole C(3)-CH_2).

diastereomer b: CIMS (100 eV) m/z (relative intensity) 352 ([M+1]$^+$, 18), 320 (41), 189 ([$C_{11}H_{13}N_2O$]$^+$, 21), 176 (68), 144 ([$C_{10}H_{10}N$]$^+$, 88), 130 ([C_9H_8N]$^+$, 100); ^{1}H NMR δ 8.24 (br s, 1H, indole NH), 7.64-7.05 (m, 5H, C(2), C(4)-C(7)H), 6.29 (br s, 1H, NH), 5.93 (br s, 1H, HNO), 5.11 (d, 1H, J=2.2Hz, OCH(OMe)), 4.88 (s, 2H, OCH_2S), 3.71 (br t, 1H, $NCHCH_2$), 3.49 (s, 3H, OCH_3), 3.32 (t, 2H, CH_2NO), 3.04 (t, 2H, indole C(3)-CH_2), 2.82-2.51 (m, 2H, $NCHCH_2$).

General Procedure of Cyclization

(1R, 13bS)-1-[(*tert*-Butyloxycarbonyl)amino]-1,2,7,8,13,13b-hexahydro-[1,6,2]oxathiazepino-[2',3':1,2]pyrido[3,4-b]indole (37a) and (1R, 13bR)-1-[(*tert*-Butyloxycarbonyl)amino]--1,2,7,8,13,13b-hexahydro-[1,6,2]oxathiazepino[2',3':1,2]pyrido[3,4-b]indole (38a)

To a cooled (-70°C) stirring solution of **32a** (2 g, 4.73 mmol) in dichloromethane (200 mL) in an argon atmosphere was added dropwise DIBAL (6.5 mL of a 1.5M solution in toluene, diluted with 20 mL dichloromethane) in 30 min. After completion of the reaction (30 min.) as was monitored by HPLC (Waters RCM 8x10, reverse phase, CH_3CN/H_2O, 60/40), the reaction mixture was cooled to -90°C and TFA (8 g, 70 mmol) was added at once. After stirring the reaction mixture for 30 minutes it was poured into 250 mL of a 0.5 N aqueous HCl solution. The organic layer was separated and the water layer washed with dichloromethane. The combined organic layers were washed with water and brine. The organic layer was dried (Na_2SO_4) and the solvent evaporated in vacuo. The residue was subjected to column chromatography (EtOAc/n-hexane/ 20/80, v/v) to yield 604 mg (34%) of **37a** and 425 mg (24%) of **38a**.

Compound 37a: Amorphous white solid, crystallization attempts failed; Rf 0.56 (EtOAc/n-hexane, 1/2, v/v); $[\alpha]^{22}_D$+7[4] (c=2.15, methanol); CIMS (100 eV) m/z (relative intensity) 376 ([M+1]$^+$, 3), 375 (M$^+$, 6), 344 (7), 232 (10), 202(31), 137(32), 135 (97), 133 (100); ^{1}H NMR (400 MHz) δ 9.98 (br s, 1H, NH), 7.45-7.42 (m, 2H, C(9)H and C(12)H), 7.18-7.05 (m, 2H, C(10)-C(11)H), 6.24 (br d, 1H, J=8.6 Hz, HNBOC), 5.26 (d (A part of AB spectrum), 1H, ^{2}J=11.4 Hz, C(4)H_A), 4.77 (dd,(B part of AB spectrum), 2H, ^{2}J=11.4 Hz and J=1.6 Hz, C(4)H_B), 4.52-4.49 (m, 1H, C(1)H), 4.13 (br s, 1H, C(13b)H), 3.78 (br d, 1H, ^{2}J=14.8 Hz, C(7)H), 3.54 (m, 1H, C(2)H), 3.07 (m, 1H, C(2)H), 2.96 (m, 1H, C(7)H), 2.82 (m, 1H, C(8)H), 2.77 (m, 1H, C(8)H), 1.52 (s, 9H, $C(CH_3)_3$); ^{13}C NMR (400 MHz) δ 156.28 (C=O), 136.59 C(12a), 132.57 C(13a), 126.00 C(8b), 121.61 C(11), 119,21 C(10), 118.00 C(9), 111.52 C(12), 107.49 C(8a), 80.45 ($OC(Me)_3$), 74.84 C(4), 73.44 C(13b), 54.89 C(1), 54.59 C(7), 32.79 C(2), 28.40 $C(CH_3)_3$, 21.14 C(8).

Compound 38a: mp 214-216°C (EtOAc/n-hexane); Rf 0.40 (EtOAc/n-hexane, 1/2, v/v); $[\alpha]^{22}_D$ +93[8] (c=1.6, methanol); UV (MeOH) λmax 224, 273.5(sh), 282, 289.5 nm; CIMS (100 eV) m/z (relative intensity) 376 ([M+1]$^+$, 19), 375 (M$^+$, 26), 320 (44), 276 (54), 232 (35), 202 (24), 186 (100), 149 (45), 57 (50); ^{1}H NMR (400 MHz) δ 8.56 (br s, 1H, NH), 7.42 (d, 1H, ^{3}J=7.7 Hz, C(12)H), 7.27 (d, 1H, J=7.7 Hz, C(9)H), 7.10-6.98 (m, 2H, C(10)-C(11)H), 5.69 (br d, 1H, J=10.4 Hz), 4.94 and 4.81 (AB spectrum, 2H, ^{2}J=9.1 Hz), 4.66 (m, 2H, C(1)H), 4.15 (br s, 1H, C(13b)H), 3.60 (m, 1H, C(7)H), 3.32 (d, 1H, J=14.5Hz, C(2)H), 3.15 (m, 1H, C(7)H), 2.97 (m, 1H, C(8)H), 2.83-2.76 (dd, 2H, C(2)H and C(8)H), 1.17 (s, 9H, $C(CH_3)_3$); Anal. Calcd. for $C_{19}H_{25}N_3O_3S$ (Mw 375.427): C, 60.78; H, 6.71; N, 11.19. Found : C, 60.62; H, 6.60; N, 11.02.

(1S, 13bR)-1-[(*tert*-Butyloxycarbonyl)amino]-1,2,7,8,13,13b-hexahydro-[1,6,2]oxathiazepino-[2',3':1,2]pyrido[3,4-b]indole (37b) and (1S, 13bS)-1-[(*tert*-Butyloxycarbonyl)amino]--1,2,7,8,13,13b-hexahydro-[1,6,2]oxathiazepino[2',3':1,2]pyrido[3,4-b]indole (38b)

In an analogous fashion **32b** (2 g, 4.73 mmol), DIBAL (2 equiv.), and TFA (8 g) gave after column chromatography (EtOAc/n-hexane/ 20/80, v/v) 621 mg (35%) of **37b** and 327 mg (18%) of **38b**.

Compound 37b: Amorpous white solid; $[\alpha]^{22}_D$ -8[3] (c=3.75, methanol). Further spectroscopic data are identical with **37a**.

Compound 38b: mp 214-216°C (EtOAc/n-hexane); $[\alpha]^{22}_D$-94[2] (c=3.8, methanol). Further spectroscopic data are identical with **38a**.

(1S, 13bR)-1-[(*tert*-Butyloxycarbonyl)amino]-10-methoxy-1,2,7,8,13,13b-hexahydro-[1,6,2]oxathiazepino[2',3':1,2]pyrido[3,4-b]indole (40) and (1S, 13bS)-1-[(*tert*-Butyloxycarbonyl)amino]--10-methoxy-1,2,7,8,13,13b-hexahydro-[1 ,6,2]oxathiazepino[2',3':1,2]pyrido[3,4-b]indole (39)

In an analogous fashion **33** (1.25 g, 2.76 mmol), DIBAL (2 equiv.) and TFA (5 mL) gave after column chromatography ($CHCl_3$) 637 mg (57%) of **40** and 268 mg (24%) of **39**.

Compound 40: mp 145-146°C (CH_2Cl_2/n-hexane); Rf 0.78 ($CHCl_3$/n-hexane, 97/3, v/v); $[\alpha]^{22}_D$-23[1] (c=5.1, methanol); CIMS (100 eV) m/z (relative intensity) 406 ($[M+1]^+$, 83), 405 (M^+, 47), 374 ($[M-CH_3O]^+$, 12), 350 (51), 320 (29), 306 (36), 262 (27), 232 (65), 216 ($[C_{12}H_{12}N_2O]^+$, 100); ^{1}H NMR δ 9.82 (br s, 1H, NH), 7.32 (dd, 1H, J=9.0Hz, C(12)H), 6.89 (s, 1H, C(9)H), 6.81 (dd, 1H, J=9.0Hz, C(11)H), 6.22 (br d, 1H, J=9.1Hz, HNBOC), 5.26 (d (A part of AB spectrum), 1H, ^{2}J=11.4Hz, C(4)H_A), 4.80 (dd (B part of AB spectrum), 1H, ^{2}J=11.4Hz, J=1.8Hz, C(4)H_B), 4.62-4.37 (m, 1H, C(1)H), 4.12 (br s, 1H, C(13b)H), 3.84 (s, 3H, OCH_3), 3.77-3.34 (m, 2H, C(7)H and C(2)H), 3.22-2.61 (m, 4H, C(7)H, C(2)H and C(8)H_2), 1.49 (s, 9H, $C(CH_3)_3$); Anal. Calcd. for $C_{20}H_{27}N_3O_4S$ (Mw 405.518) :C, 59.24; H, 6.71; N, 10.36. Found: C, 59.11; H, 6.74, N, 10.31.

Compound 39: mp 184-220°C (decomp.); Rf 0.53 ($CHCl_3$/MeOH, 97/3, v/v); $[\alpha]^{22}_D$-55[3] (c=4.05, methanol); CIMS (100 eV) m/z (relative intensity) 406 ($[M+1]^+$, 57), 405 (M^+, 44), 350 (51), 320 (17), 306 (50), 262 (32), 232 (17), 216 ($[C_{12}H_{12}N_2O]^+$, 100);^{1}H NMR δ 8.46 (br s, 1H, NH), 7.17 (d, 1H, J=9.0Hz, C(12)H), 6.91-6.71 (m, 2H, C(9)H and C(11)H), 5.70 (br d, 1H, HNBOC), 4.97 and 4.81 (AB spectrum, 2H, ^{2}J=9.0 Hz, C(4)H_2), 4.72-4.56 (m, 1H, C(1)H), 4.14 (br s, 1H, C(13b)H), 3.86 (s, 3H, OCH_3), 3.61 (m, 1H, C(7)H), 3.34 (m, 1H, C(2)H), 3.13 (m, 1H, C(7)H), 2.94 (m, 1H, C(8)H), 2.83-2.76 (m, 2H, C(2)H and C(8)H), 1.20 (s, 9H, $C(CH_3)_3$); Anal. Calcd. for $C_{20}H_{27}N_3O_4S$ (Mw 405.518) :C, 59.24; H, 6.71; N, 10.36. Found: C, 58.99; H, 6.62, N, 10.27

Removal of the BOC-group

(1R,13bS)-1-Amino-1,2,7,8,13,13b-hexahydro-[1,6,2]oxathiazepino[2',3' :1,2]pyrido[3,4-b]indole (41a)

A solution of **37a** (604 mg, 1.61 mmol), chlorotrimethylsilane (351 mg, 3.22 mmol) and NaI (483. mg, 3.22 mmol) in acetonitrile (200 mL) was stirred at room temperature during 3 hours. The solvent was evaporated in vacuo, the residue was dissolved in dichloromethane and subsequently washed with water and brine. The organic layer was dried (Na_2SO_4) and the solvent evaporated in vacuo. The residue was subjected to column chromatography (EtOAc/n-hexane, 1/1, v/v) to yield 423 mg (95%) of **42a**. mp 147-149°C (CH_2Cl_2/n-hexane); Rf 0.24 ($CHCl_3$/MeOH, 97/3, v/v); $[\alpha]^{22}_D$-28[8] (c=1.7 ,methanol); CIMS (100 eV) m/z (relative intensity) 276 ($[M+1]^+$, 27), 275 (M^+, 20), 232 (21), 203 (27), 202 (32), 186 (91), 172 (40), 171 (100), 169 (35), 144 (35); ^{1}H NMR (400 MHz) δ 10.05 (br s, 1H, NH), 7.46 (d, 1H, J=7.7 Hz, C(12)H)), 7.32 (d, 1H, J=8.1 Hz, C(9)H), 7.15-7.04 (m, 2H, C(10)-C(11)H), 4.93 (br s, 2H, C(4)H_2), 3.71-3.60 (br d, 3H, C(13b)H, C(1)H and C(7)H), 3.13-2.77 (m, 5H, C(2)H_2, C(7)H and C(8)H_2), 1.59 (br s, 2H, NH_2); ^{13}C NMR (400 MHz) δ 135.78 C(12a), 133.73 C(13a), 126.08 C(8b), 121.28 C(11), 118.98 C(10), 118.06 C(9), 110.98 C(12), 106.79 C(8a), 73.08 C(4), 69.02 C(13b), 59.70 C(1), 54.98 C(7), 37.97 C(2), 20.81 C(8); Anal. Calcd. for $C_{14}H_{17}N_3OS$ (Mw 275.374): C, 61.06; H, 6.22; N, 15.26. Found: C, 60.99; H, 6.10; N, 15.11.

(-)-Debromo-eudistomin L ((-)-1e)

Following the same procedure as described for **37a** with **38b** (327 mg, 0.87 mmol), chlorotrimethylsilane (190 mg, 1.74 mmol) and NaI (260 mg, 1.74 mmol) gave after column chromatography ($CHCl_3$/MeOH, 98/2, v/v) 225 mg (94%) of **1e**.: Amorphous white solid; Crystallization attempts were unsuccessful; Rf 0.06 ($CHCl_3$/MeOH, 97/3, v/v); $[\alpha]^{22}_D$ -115[3] (c=3.0, methanol); UV (MeOH) λmax 223, 273.5(sh), 282, 289 nm; CIMS (100 eV) m/z (relative intensity) 276 ($[M+1]^+$, 3), 275 (M^+, 1), 232 (11), 231 (14), 212 (19), 211 (21), 203 (82), 202 (100), 186 (34), 172 (22), 171 (32), 169 (37), 144 (44); ^{1}H NMR (400 MHz) δ 8.33 (br s, 1H, NH), 7.47 (d, 1H, J=7.6 Hz, C(12)H), 7.32 (d, 1H, J=8.0 Hz, C(9)H), 7.17-7.13 (m, 2H, C(10)-C(11)H), 4.92 and 4.80 (AB spectrum, 2H, ^{2}J=9.0 Hz, C(4)H_2), 4.08 (br s, 1H, C(13b)H), 3.57 (m, 1H, C(7)H), 3.51 (br s, 1H, C(1)H), 3.31 (d, 1H, ^{2}J=14.4Hz, C(2)H), 3.13 (m, 1H, C(7)H), 2.94 (m, 1H, C(8)H), 2.84 (dd, 1H, ^{2}J=14.3Hz, J=5.7Hz, C(2)H), 2.81 (m, 1H, C(8)H), 1.85 (br s, 2H, NH_2); ^{13}C NMR (400 MHz) δ 136.90 C(12a), 130.67 C(13a), 126.32 C(8b), 122.13 C(11), 119.82 C(10), 118.26 C(9), 111.16 C(12), 110.96 C(8a), 71.36 C(4), 69.93 C(13b), 53.98 C(7), 50.73 C(1), 33.99 C(2), 20.67 C(8).

(1S,13bR)-1-Amino-1,2,7,8,13,13b-hexahydro-[1,6,2]oxathiazepino[2',3' :1,2]pyrido[3,4-b]indole (41b)

Following the same procedure as described for **37a** with **37b** (621 mg, 1.66 mmol), chlorotrimethylsilane (362 mg, 3.32 mmol) and NaI (500 mg, 3.32 mmol) gave after column

chromatography (EtOAc/n-hexane, 1/1, v/v) 404 mg (89%) of **41b**. mp 146-149°C (CH_2Cl_2/n-hexane); $[\alpha]^{22}_D$ +23[2] (c=3.8, methanol). Further spectroscopic data are identical with **41a**.

(+)-Debromo-eudistomin L ((+)-1e)
Following the same procedure as described for **37a** with **38a** (425 mg, 1.13 mmol), chlorotrimethylsilane (246 mg, 2.26 mmol) and NaI (340 mg, 2.26 mmol) gave after column chromatography ($CHCl_3$/MeOH, 98/2, v/v) 303 mg (98%) of (+)-**1e**. Amorphous white solid; Crystallization attempts were unsuccessful; $[\alpha]^{22}_D$+111[4] (c=2.1 ,methanol). Further spectroscopic data are identical with (-)-**1e**.

(1S, 13bS)-1-Amino-10-methoxy-1,2,7,8,13,13b-hexahydro-[1,6,2]oxathiazepino[2',3':1,2]-pyrido[3,4-b]indole ((-)-1f)
Following the same procedure as described for **37a** with **39** (155 mg, 0.38 mmol), chlorotrimethylsilane (83 mg, 0.76 mmol) and NaI (114 mg, 0.76 mmol) gave after column chromatography ($CHCl_3$/MeOH, 99/1, v/v) 111 mg (95%) of (-)-**1f**.; Amorphous white solid; Crystallization attempts were unsuccessful; Rf 0.36 ($CHCl_3$/MeOH, 93/7, v/v); $[\alpha]^{22}_D$-76[6] (c=2.7, methanol); CIMS (100 eV) m/z (relative intensity) 306 ($[M+1]^+$, 13), 276 (37), 232 (100), 216 ($[C_{12}H_{12}N_2O_2]^+$, 19), 201 (49), 200($[C_{12}H_{12}N_2O]^+$, 22), 199 (20), 174 (30); ^{1}H NMR (400 MHz) δ 8.22 (br s, 1H, NH), 7.19 (d, 1H, J=9.0Hz, C(12)H), 6.92-6.76 (m, 2H, C(9)H and C(11)H), 4.93 and 4.81 (AB spectrum, 2H, ^{2}J=9.0 Hz, C(4)H_2), 4.06 (br s, 1H, C(13b)H), 3.83 (s, 3H, OCH_3), 3.56 (m, 1H, C(7)H), 3.42 (m, 1H, C(1)H), 3.27 (m, 1H, C(2)H), 3.09 (m, 1H, C(7)H), 2.95 (m, 1H, C(8)H), 2.83 (dd, 1H, C(2)H), 2.81 (m, 1H, C(8)H), 1.87 (br s, 2H, NH_2).

(1S, 13bR)-1-Amino-10-methoxy-1,2,7,8,13,13b-hexahydro-[1,6,2]oxathiazepino[2',3':1,2]-pyrido[3,4-b]indole (42)
Following the same procedure as described for **37a** with **40** (110 mg, 0.27 mmol), chlorotrimethylsilane (59 mg, 0.54 mmol) and NaI (81 mg, 0.54 mmol) gave after column chromatography (EtOAc/n-hexane, 40/60, v/v) 65 mg (78%) of **42**. Crystallized from CH_2Cl_2/n-hexane: mp 104-105°C; Rf 0.24 ($CHCl_3$/MeOH, 93/7, v/v); $[\alpha]^{22}_D$+3°(c=2.0, methanol); CIMS (100 eV) m/z (relative intensity) 306 ($[M+1]^+$, 48), 262 (30), 233 (38), 232 (54), 216 ($[C_{12}H_{12}N_2O_2]^+$, 100), 201 (95), 200($[C_{12}H_{12}N_2O]^+$, 51), 199 (35); ^{1}H NMR (400 MHz) δ 9.87 (br s, 1H, NH), 7.23 (d, 1H, J=9.0Hz, C(12)H)), 6.94-6.73 (m, 2H, C(9)H and C(11)H), 4.94 (br s, 2H, C(4)H_2), 3.87 (s, 3H, OCH_3), 3.77-3.51 (br m, 3H, C(13b)H, C(1)H and C(7)H), 3.13-2.77 (m, 5H, C(2)H_2, C(7)H and C(8)H_2), 1.69 (br s, 2H, NH_2).

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CHAPTER 8

Structure -activity relationship (SAR) studies

"Structure-activity relationship studies of N-hydroxy(alkoxy)tryptamine derivatives on the serotonin- and tryptamine receptors.", Pedro H.H. Hermkens, Jan H. v. Maarseveen, Martin Th. M. Tulp, Hans W. Scheeren, Chris G. Kruse, *in preparation.*

"Structure-actvity relationship studies of eudistomin derivatives on antitumour and antiviral activities." Pedro H.H. Hermkens, Jan H. v. Maarseveen, Peter Lelieveld, Eric De Clercq, Jan Balzarini, Chris G. Kruse, Hans W. Scheeren, *in preparation.*

Chapter 8.1

STRUCTURE-ACTIVITY RELATIONSHIP (SAR) STUDIES OF N-OXY-TRYPTAMINES AND N-OXY-β-CARBOLINES ON BENZODIAZEPINE-, SEROTONIN- AND TRYPTAMINE-RECEPTORS

8.1.1 Introduction

Tetrahydro-β-carboline (THBC) and β-carboline derivatives have been detected in human and rat tissues and body fluids[1]. In man they were mainly found in plasma and platelets. Considerable differences in concentration of some β-carbolines were reported, even by the same group of investigators, which could be due to artifactual formation of β-carbolines during the work-up procedures. Some doubt still exists as to their physiological occurrence, because these compounds are very easily formed from formaldehyde and indoleamines. Anyhow, despite the question whether the β-carbolines are endogenous substances, they are interesting because they show a very broad pharmacological profile.

Monoamine oxidase (MAO) is the principal enzyme concerned with the inactivation of serotonin (5-HT) and other neurotransmitters. It has been demonstrated[2] that β-carbolines and THBC are potent and selective MAO-inhibitors. It has also been observed[3] that β-carbolines increase 5-HT concentrations probably by 5-HT reuptake inhibition. Furthermore, β-carbolines show variable affinity for the benzodiazepine-[4], serotonin-[5] and tryptamine[6] receptors.

It is obvious that these compounds are of major pharmacological interest. Because virtually nothing is known about the pharmacology of N(2)-hydroxy(alkoxy)-tetrahydro- and N(2)-oxo-β-carboline derivatives and the corresponding N-hydroxy(alkoxy)tryptamines, from which they are prepared, it was interesting to subject these compounds to a first pharmacologocal screening. In our view the benzodiazepine-, serotonin- and tryptamine-receptors were the most logical choice.

Benzodiazepine receptor

It is generally accepted that the postsynaptic (γ-aminobutyric acid) $GABA_A$-receptor is a tetrameric membrane protein complex, consisting of very similar protomers (subunits). The quaternary structure enables the formation of a Cl^- ionophore that can close and open in response to small conformational changes in the subunits. The subunits are proposed to have at least three domains with different functions *i.e.* GABA-binding sites, binding sites for benzodiazepines (BZ) or β-carbolines and sites which bind picrotoxin and barbiturates (Figure 1)[7,8]. At receptor level *in vitro* studies have shown that BZ agonists enhance the binding of GABA to its receptor, inverse agonists inhibit GABA binding while antagonists, which reverse both of these effects, have no influence on GABA binding. The reverse effect, that is binding of the ligands to the BZR in the presence of

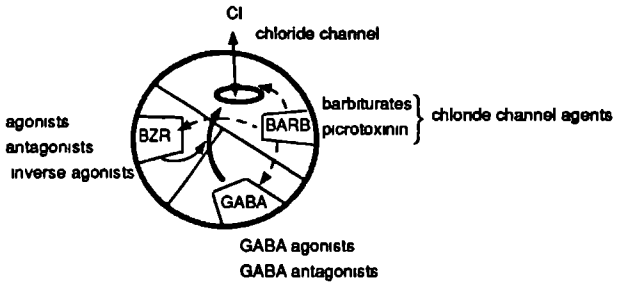


Fig 1 Schematic model of a subunit of the postsynaptic $GABA_A$ receptor
The main funtion of the complex is the GABA-induced gating (opening) of the Cl^- channel (large arrow), with the regulation of this gating process by BZ agonists and inverse agonists (medium arrow), and regulation of Cl channel properties by ligands like picrotoxin and barbiturates (dotted arrow)

GABA, follows the same pattern; the affinity of agonists is enhanced by GABA, the affinity of inverse agonists is decreased in the presence of GABA, and there is no effect of GABA on the affinity of antagonists. Some barbiturates and other agents, likewise thought to interact directly with chloride channels, enhance the affinity of agonists to the BZR[9]. It has been postulated that these differences in activity are due to conformational changes effected at the BZR by the various ligands (Figure 2)[7,8,10,11]. Stimulation of the GABA receptor induces a conformational change which

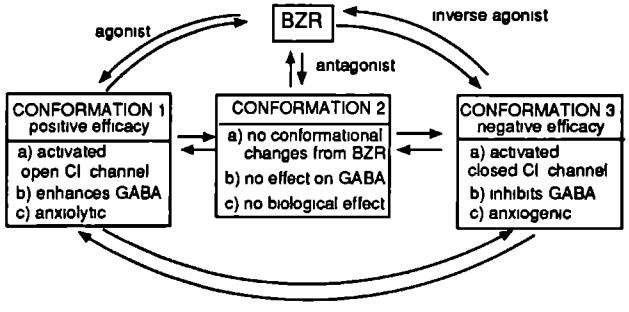


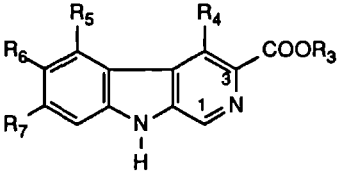
Fig. 2 Proposed model for interactions of Agonist, Antagonist and Inverse Agonist Ligands at the BZR

triggers the opening of chloride channels; the benzodiazepine receptor is suggested to operate as a coupling unit between the GABA receptor and the chloride channel. Benzodiazepine agonists enhance, inverse agonists reduce the coupling function.

The BZR ligands are thought to comprise a continuous spectrum of agents with a graduated range of pharmacological efficacies at the receptor: (1) full inverse agonists (negative efficacy; anxiogenic/convulsant), (2) partial inverse agonists (intermediate negative efficacy; proconvulsant), (3) pure antagonists (nil efficacy; antagonism toward the other classes), (4) partial agonists, and (5) full agonists (positive efficacy; anxiolytic/anticonvulsant)[11,12]. This spectrum of differing efficacy

has been most clearly demonstrated in the β-carbolines (Chart I)[12-14]. It was proposed that such

Chart I



1	R_3	R_4	R_5	R_6	R_7	type of efficacy
a	Et	CH_2OCH_3	H	OBn	H	5
b	Et	CH_2OCH_3	OBn	H	H	4
c	Et	Me	$OCH(Me)_2$	H	H	3
d	Et	H	H	H	H	2
e	Me	Et	H	OMe	OMe	1

β-carbolines, which were isolated from human urine and brain tissue compose part of the endogenous ligand structure of the BZR[4,15,16].

So the β-carboline structure might become an important basis for the design of new benzodiazepine-related drugs. Studies on structure-activity relationships (SAR)[17] have demonstrated that 3,4-dihydro-, or tetrahydro-β-carbolines showed decreased activity compared to the corresponding fully aromatic compounds. Introduction of functionalities at positions C(1) and/or N(9) results in loss of activity. At position 3 the presence of ester groups is fundamental for an optimal drug-receptor interaction. The 3,4-dihydro-N(2)-oxide analogue of **1d** causes a drop in activity, though the fully aromatic analogue still preserves an appreciable affinity towards the receptor[18].

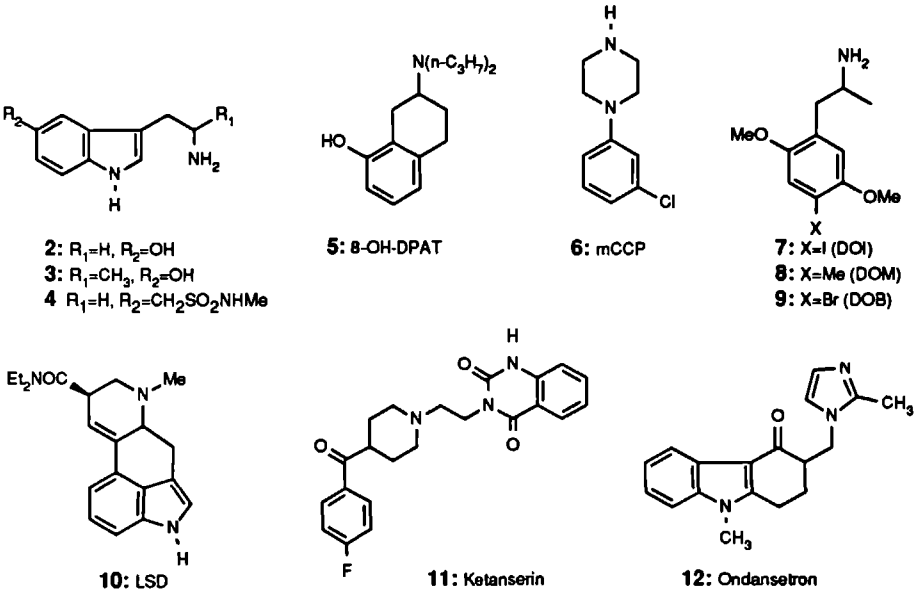
In order to study the effect of the presence of functional groups at C(1) and/or N(2) on the activity of β-carbolines we prepared 1,3-disubstituted N(2)-oxy-1,2,3,4-tetrahydro-β-carbolines (chapters 5 and 6) and 1, and/or 3-substituted N(2)-oxo-3,4-dihydro-β-carbolines (chapter 3) and investigated their affinity towards the benzodiazepine receptor.

Serotonin receptor

Appetite, memory, thermoregulation, sleep, sexual behaviour, anxiety, depression, and hallucinogenic behaviour are some of the processes that have been linked with the neurotransmitter serotonin (5-hydroxytryptamine=5-HT;**2**, Chart II). With the recent discovery of multiple populations of central serotonin binding sites (*i.e.* $5\text{-}HT_{1A}$, $5\text{-}HT_{1B}$, $5\text{-}HT_{1C}$, $5\text{-}HT_{1D}$, $5\text{-}HT_2$ and $5\text{-}HT_3$ sites) has come a renewed interest in this neurotransmitter, particularly in light of the possibility that its interaction with different types of central sites might explain its various actions.[19]

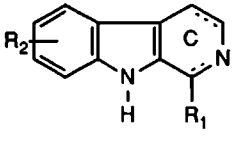
One of the most significant problems facing serotonin research today is a lack of site-selective agonists and antagonists; a continued lack of such tools will surely retard further advances in this

Chart II



field. By adapting the chemical struture of 5-HT, scientists have synthesized analogous molecules, which act by triggering or blocking only specific types of receptors. This selectivity of 5-HT drugs means that they have no apparent side-effects. Some of the structural classes currently being explored as central 5-HT agonists and antagonists are indolylalkylamines (*e.g.* 5-HT;**2**, 2-Me-5-HT;**3**, sumatriptan;**4**), aminotetralins (*e.g.* 8-OH-DPAT;**5**), arylpiperazines (*e.g.* mCCP;**6**), phenylalkylamines (*e.g.* DOI;**7**, DOM;**8**, and DOB;**9**), ergolines (*e.g.* LSD;**10**), alkylpiperidines (*e.g.* ketanserin;**11**) and carbazole derivatives (*e.g.* ondansetron;**12**) (Chart II)[19].

Our first goal is to compare the indolylalkylhydroxyl(alkoxy)amines (chapter 2) with indolylalkylamines in order to study the influence of the oxygen adjacent to the nitrogen. Secondly, because the β-carbolines are composed of an indolylalkylamine backbone, and because several derivatives (harmine, harmaline and related compounds *e.g.* **13**)[5] showed moderate affinity for the



13

R_1=H, CH_3

R_2=OH, OMe

Ring C=Aromatic or Not-Aromatic

5-HT receptor we also studied the affinity and selectivity of several N-oxy-1,2,3,4-tetrahydro-β-carbolines (chapters 5 and 6).

Tryptamine receptor

The functional role of tryptamine in the CNS is not clearly defined at the present time. Recently it has been suggested that the most consistent feature of this amine is the potentiation of 5-HT, presumably via a postsynaptic action. However, a synaptic action independent from 5-HT cannot be excluded at this time. It has been clearly demonstrated by competition experiments that the tryptamine binding sites are distinct from 5-HT or catecholamine receptors.

β-Carbolines of the harman-famile (*e.g.* **13**) showed a high affinity for the tryptamine receptor[6]. Therefore, it was of interest to examine whether N(2)-oxy-β-carbolines (chapters 5 and 6) has any affinity for this receptor.

8.1.2 Receptor Binding Assays

Receptor binding assays were performed as reported earlier (see Table I) at Duphar Research Laboratories, Weesp. Ability of the compounds to displace the [^{3}H]-ligand from the tissue homogenates was than measured, as an indication of their affinity for the corresponding receptors.

Table I. Receptor binding methodology.

Receptor	Tissue[a]	[^{3}H]-Ligand	Reference
benzodiazepine	total brain	diazepam	20
5-HT$_{1A}$	frontal cortex	8-OH-DPAT	21
5-HT$_{1B}$	frontal cortex	serotonin[e]	22
5-HT$_{1C}$	choriod plexus[b]	serotonin	23
5-HT$_{1D}$	n.caudate[c]	serotonin[e]	24
5-HT$_2$	frontal cortex	spiperone	25
5-HT$_3$	nb-g cells[d]	GR 38032F	26[f]
tryptamine	cerebal cortex	tryptamine	27
GABA$_A$	cerebellum	DH-Muscimol	28

a) Rat. b) Pig c) Bovine d) Cultivated mouse neuroblastoma-glioma cells
e) In these assays 3 10^{-8}M unlabelled 8-OH-DPAT and 3 10^{-8}M DOI are added to the incubation medium in order to block 5-HT$_{1A}$ and 5-HT$_{1C}$ receptors, respectively
f) Method identical to this reference, except [^{3}H] GR 38032F was used as ligand, rather than [^{3}H] ICS205,930

N-oxy-tryptamines

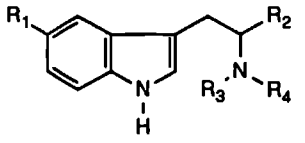
Over the past two decades, considerable synthetic and pharmacological effort has been directed to 'fine-tuning' studies of hallucinogenic phenethylamines and tryptamines.

The basic goal of this research was to gain a better understanding of the structure-activity relationships of these substances with respect to the serotonin- and tryptamine-receptors. Within the indolylalkylamine family the major chemical alterations have involved changes in the amine alkyl substituents. This has been partly due to the relative ease of synthetic manipulations at this site and also because substantial biological differences can be attributed to even minor changes in these substituents. Only recently a new selective drug has been introduced, which is an 5-HT analogue

with a different, aromatic substituent. Sumatriptan (4), a 5-HT_1-like agonist, looks promising in treatment of migraine[29].

In contrast, little is known about N-oxy tryptamine derivatives. Earlier publications reported that 1-(3-indolyl)-2-hydroxylaminopropane causes no significant inhibition of monoamine oxidase while 1-(3-indolyl)-2-aminopropane is a potent inhibitor of this enzyme[30]. However, the observation that in the rabbit and the guinea-pig liver, tryptamine was converted in β-(3-indolyl)ethylhydroxylamine[31] promted us to investigate the N-oxy tryptamines. For this reason we measured the affinity of several N-hydroxy- and N-methoxytryptamine derivatives (chapter 2) for the serotonin- and tryptamine-receptors and compared these values with those of the corresponding tryptamine- and N-methylated tryptamine-derivatives (Table II). We considered three classes *i.e.* N-substituted tryptamines (entries 1-5), N-substituted 5-methoxytryptamines (entries 6-9) and α-substituted tryptamines (entries 10-13).

Table II. Radioligand Binding Data for tryptamine and N-oxy-tryptamine derivatives[a].



Entry	R_1	R_2	R_3	R_4	affinity, pKi						
					5-HT_{1A}	5-HT_{1B}	5-HT_{1C}	5-HT_{1D}	5-HT_2	5-HT_3	trypt
1	H	H	H	H	7 10	7 32	7.78	7 24	5 54	<5.14	8 21
2	H	H	Me	H	7.21	6 95	6 69	7 18	5 69	4.78	6 08
3	H	H	Me	Me	6 85	6 36	6 47	6 34	5 80	5.43	5 50
4	H	H	H	OH	5 4	6 19	7 11	<6 2	<6 2	<6 11	6 66
5	H	H	H	OMe	4 83	4 64	4.12	3 68	4 19	<6 11	5 25
6	OH	H	H	H	8 38	8 61	8 37	8 46	5 89	6 44	5 81
7	OMe	H	H	H	8 51	7 98	8 05	8 30	5 94	<5 14	6 42
8	OMe	H	Me	Me	8 20	7.07	7 69	7.41	5 64	<5 14	<5 09
9	OMe	H	H	OH	7 00		7 20	6 90	<5 2	<6 1	<6 1
10	H	Me[b]	H	H	5 78	5 65	7 27	5 40	5 42	5.13	7 50
11	H	Me[b]	H	OH	<6 2	<6 2	5 23	<6 2	<5 2	<6 11	4.66
12	H	Me[b]	H	OMe	<6 2	<6 2	4 44	<6 2	<6 2	<6 1	4 1
13	H	Ph[b]	H	OH	<6 2		<6 3		4 59		<6 1

a) All values represent the mean of two or three determinations b) racemic mixtures

In the first class, methyl substitution at the nitrogen of tryptamine reduces the affinity for the tryptamine receptor substantially, while for the serotonin 5-HT_1 receptors there is a moderate decrease in affinity (entries 1-3). Therefore the decrease in affinity of N-hydroxytryptamine for the tryptamine receptor (entry 4) can be ascribed to steric hindrance. Since the affinity of the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} receptors is significant lower than the affinity of the N-methyl analogues electronic influences must play an important role. Surprisingly, the affinity for the 5-HT_{1C} receptor is hardly affected and therefore N-hydroxytryptamine is a relatively selective ligand for the 5-HT_{1C}

receptor. At this moment it is not known whether this ligand has agonistic or antagonistic properties at this receptor. N-methoxytryptamine (entry 5) has no affinity for the serotonin and tryptamine receptors. Especially, the lost in affinity for the 5-HT_{1C} receptor is of interest, because electronic rather than steric effects must be the reason for this.

In the second class, serotonin and 5-methoxytryptamine show high affinities for the 5-HT_1 receptors (entries 6,7). Nitrogen alkylation (entry 8) has no influence on the affinity for the 5-HT_{1A} receptor, while there is a noticeable decrease in affinity for the remaining 5-HT_1 receptors. The decrease in affinity of 5-methoxy-N-hydroxytryptamine (entry 9) for the 5-HT_{1A} receptor and for the remaining 5-HT_1 receptors is probably the result of electronic effects. Selectivity for this ligand is out of the question, because the affinities for the 5-HT_1 receptors are comparable.

In the third class, we see that α-methyltryptamine (entry 10) has a selectivity for the 5-HT_{1C}- and the tryptamine receptor. For the N-Hydroxy- and N-methoxy analogues the affinity for these receptors is abolished (entries 11-12). Whether electronic or steric factors are responsible for this decrease is yet unknown. α-Phenyl-N-hydroxytryptamine (entry 13) likewise shows no affinity for the serotonin- and tryptamine-receptors.

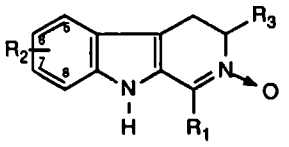
In conclusion, it can be said that introduction of a hydroxy or methoxy function at the nitrogen decreases the affinity for the tryptamine receptor due to steric factors. For the 5-HT_1 receptors there is a decrease of affinity due to electronic factors. These effects are stronger for the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1D} than for the 5-HT_{1C} receptor. The electron density on the nitrogen decreases in the row N-methyltryptamine > tryptamine ≈ α-methyltryptamine > N-hydroxytryptamine > N-methoxytryptamine[32]. With decreasing electron density also the net contribution of this nitrogen for receptor binding will decrease, though the 5-HT_{1C} receptor is the least critical. N-hydroxytryptamine is a relatively selective substrate for this receptor.

N(2)-Oxo-1-and/or 3-substituted-3,4-dihydro-β-carbolines (Table III).

The earlier observed[18] considerable affinity of 2-oxo-3-(ethoxycarbonyl)-3,4-dihydro-β-carboline (Entry 1) for the BZR did us decide to study this class of compounds (chapter 3.1) in more detail. Therefore we prepared the 5-chloro- and 6-chloro analogues of this compound (Entries 2,3). The observed affinities (pKi's of 7.67 and 7.81, respectively) were very high for this type of not-aromatic dihydro-β-carbolines. Because of an inconsistency in the SAR of the nitrile cycloadducts *(vide infra)* we studied these compounds in more detail. It had already been reported[33] that these nitrones very easily decompose to 3-(ethoxycarbonyl)-β-carboline (β-CCE; **1d**) of which it is known that it is a potent inhibitor of the specific binding of [^{3}H]-diazepam to its brain receptors. Therefore it seemed obvious to determine if β-CCE formation might take place during the formulation of the samples. By means of a quantitative TLC determination method we were able to establish that decomposition of the nitrones (entries 1-3) takes place indeed and that the resulting β-CCE analogues were responsible for the observed affinity and not the parent compounds.

The 3-methyl nitrones (entries 4,5) did not decompose to the corresponding aromatic β-carbolines and no affinity was observed for the BZR. This is in agreement with known SAR for this receptor

Table III. Radioligand Binding Data fot N(2)-oxo-3,4-dihydro-β-carbolines[a].



Entry	R_1	R_2	R_3	affinity pKi		
				BZ	5-HT_{1A}	5-HT_2
1	H	H	COOEt	6.68[b]	<6.2	<6.3
2	H	5-Cl	COOEt	7.67[b]		<5.3
3	H	6-Cl	COOEt	7.81[b]	<6.2	<5.3
4	H	H	CH_3	<6.1	<6.2	<5.2
5	Ph	H	CH_3	<6.1	<6.2	<5.2

a) All values represent the mean of two or three determinations b) The observed affinity is a result of formation of the corresponding aromatic β-carboline during the formulation.

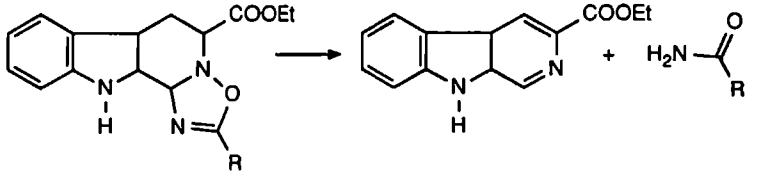
i.e. the aromatic character and the presence of an ester group at position 3 are essential.

All the nitrones (entries 1-5) showed no specific inhibition of the binding of the [^{3}H]-ligand to the 5-HT_{1A} and 5-HT_2 receptor.

Nitrile cycloadducts (Table IV).

Initially, very high affinities for the BZR were found for some of the nitrile cycloadducts (chapter 3.2)(entries 1-16). However, an inconsistency in the SAR made us suspicious. Therefore we examined the adducts on their stability. We found that that the cycloadducts partially decomposed to give β-CCE and the corresponding amides when they were kept at 80°C for 2 days (Scheme I)[34]. By

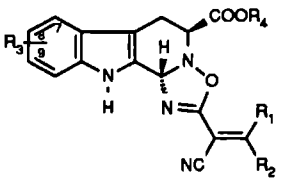
Scheme I



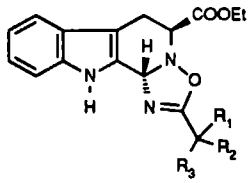
means of a quantitative TLC determination method we established that β-CCE formation took also place during the formulation of the samples. We were able to measure that the decomposition of the cycloadducts resulting in β-CCE analogues was responsible for the observed affinity and not the parent compounds.

All the adducts (entries 1-16) showed no affinity for the 5-HT_{1A} and 5-HT_2 receptor (pKi<6.2 and pKi<5.3, respectively).

Table IV. Nitrile cycloadducts



Entry	R_1	R_2	R_3	R_4
1	H	Ph	H	Et
2	H	Ph	9-OMe	Et
3	H	Ph	H	Bn
4	H	p-Cl-Ph	H	Et
5	H	p-F--Ph	H	Et
6	H	Ph	H	Me
7	H	Ph	7-Cl	Et
8	H	Ph	6-Cl	Et
9	SMe	SMe	H	Et
10	Ph	Ph	H	Et
11	H	2-furanyl	H	Et



Entry	R_1	R_2	R_3
12	Me	Me	CN
13	Bn	Bn	CN
14	Me	Me	EtOOC
15	Me	Me	NO_2
16		$-(CH_2)_4-$	CN

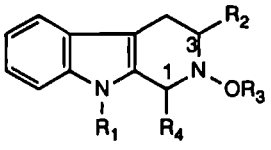
1- and/or 3-Substituted N-oxy-1,2,3,4-tetrahydro-β-carbolines (Table V).

Subsequently, we investigated the hitherto unknown N(2)-oxy-1,2,3,4-tetrahydro-β-carbolines (chapter 5) presented in Table V on their receptor affinity.

For all the compounds tested (entries 1-27) there was no high affinity for the binding-sites of the benzodiazepine (pKi<6.1), 5-HT_{1A} (pKi<6.2), 5-HT_{1B} (pKi<6.2), 5-HT_2 (pKi<5.2) and tryptamine receptor (pKi<6.1). No specific inhibition of [^{3}H]-serotonin binding at the 5-HT_{1C} receptor was observed in the case of entries 2, 10 and 11.

Although no specific inhibition was observed for [^{3}H]-diazepam binding, we observed that some of the β-carbolines with a phenyl group at position 1 (entries 3, 4, 5 , 10 and 11), caused a potentiation of binding of this ligand (See Figure 3). This is a known phenomenon in the presence of a GABA agonist. Although, the level of stimulation of the compounds of entry 3, 4, 5 and 10 is mediate, that of *trans* 1-phenyl-2-hydroxy-3-phenyl-1,2,3,4-tetrahydro-β-carboline (entry 11) is comparable with that of GABA (see figure 3). This potentiation of a BZ agonist by β-carbolines has not been described before. Our first hypothesis was that these types of β-carbolines therefore should act as GABA agonists. However, they did not show any specific inhibition of [^{3}H]-DH-muscimol binding, the ligand of the the GABA receptor. Therefore the affinity site must lay somewhere else. A possibility is that this type of β-carbolines have an affinity for the barbiturate receptor, as some barbiturates are known to enhance the affinity for agonists to the BZR[9]. Another rational is that they directly interfere with the chloride channel protein or picrotoxinin sites. Although, the mechanism of action at this point is uncertain yet, we believe that a more detailed study is of interest.

Table V. 1- and/or 3-Substituted-N-oxy-1,2,3,4-tetrahydro-β-carbolines.



Entry	R_1	R_2	R_3	R_4	C_1-C_3	Entry	R_1	R_2	R_3	R_4	C_1-C_3
1	H	COOEt	H	Ph	cis	15	H	COOEt	H	2-thienyl	trans
2					trans	16				3,4,5-$C_6H_2(OMe)_3$	cis
3			Me		trans	17					trans
4			i-Pr		trans	18		Me			cis
5			n-Bu		cis	19					trans
6					trans	20		COOEt		Me	cis
7		$CONHC_3H_5$	H		cis	21					trans
8					trans	22				n-Pr	cis
9	Me	CONHMe			trans	23					trans
10	H	Ph			cis	24				Benzyl	cis
11					trans	25					trans
12		Me			cis	26		Ph		Me	cis
13					trans	27		H		Me	
14		COOEt		2-thienyl	cis						

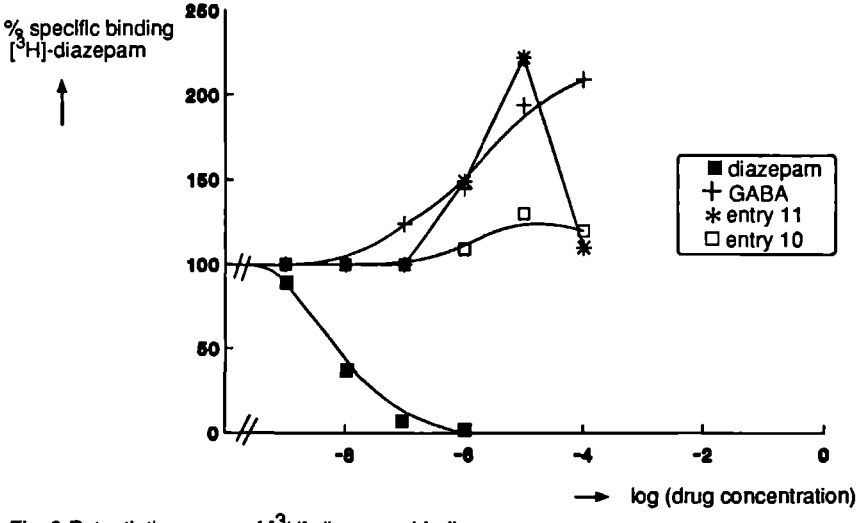


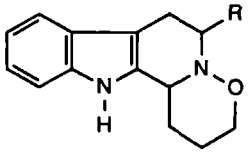
Fig. 3 Potentiation curve of [^{3}H]-diazepam binding

An application of this observed potentiation might be that the doses of the benzodiazepine constituent in a combination-drug of these β-carbolines and bezodiazepines, would lead to a decrease of the required amount of the tranquilizer.

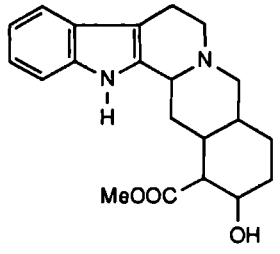
Tetracyclic N-oxo-β-carbolines (Table VI)

Although, the N-carbon tetracyclic-β-carboline is a structural feature present in many indole alkaloids (*i e* yohimbine (**17**) and corynantheine (**18**)), with interesting pharmacological activities, the N-oxo analogues **14-16** show no specific inhibition of the [^{3}H]-ligand binding at the

Chart II



14: R=COOEt, H(6)α, H(12b)α
15: R=COOEt, H(6)α, H(12b)β
16: R=H



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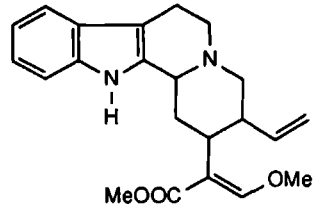

18

Table VI Tetracyclic N-oxo β carbolines

Compound	affinity, pKi							
	Benzod	5 HT_{1A}	5-HT_{1B}	5 HT_{1C}	5 HT_{1D}	5 HT_2	5 HT_3	tryptamine
14	<6 1	5 0	<6 2		<6 2	<5 2	<6 1	<6 1
15	<6 1	4 47	<6 2	<6 3	<6 2	<5 2	<6 1	<6 1
16	<6 1	5 12	<6 2	<6 3	<6 2	<5 2	<6 1	<6 1

benzodiazepine, 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D}, 5-HT_2, 5-HT_3 and tryptamine receptors (Table VI) A direct comparison between the N-oxo and N-carbon analogues is at this point impossible, because the activities of the N-carbon analogues of **14-16** are unknown

As it is possible now to prepare this class of tetracyclic N-oxo compounds (see chapter 6), it is of importance that N-oxo compounds will be prepared from which the N-carbon analogues have interesting pharmacological activities For instance yohimbine (**17**) exerts a complex pattern of pharmacological actions that include blockade of α-adrenergic and serotonin (5-HT) receptors

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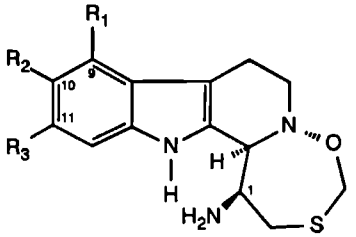
Chapter 8.2

STRUCTURE ACTIVITY RELATIONSHIPS OF EUDISTOMIN ANALOGUES WITH REGARD TO THEIR ANTITUMOUR AND ANTIVIRAL ACTIVITY

8.2.1 Introduction

It is of great importance to enrich the current chemotherapy, in particular the antiviral and antitumour drugs with new chemical structures. Hereby are of special interest compounds which derive their action from new and specific points of interaction with the biological substrate. Recently, a new class of compounds -eudistomins- has been isolated which has a realistic possibility to reach this goal.

Eudistomins A-Q were extracted from the colonial tunicate *Eudistoma olivaceum*, collected in the shallow water in Mexico, Belize and Florida[1]. Crude extracts of all the *E. olivaceum* samples collected displayed activity against *Herpes Simplex* virus, type I (HSV-1). Four groups of eudistomins have been isolated, including simple β-carbolines (D, J, N, and O), pyrrolyl-β-carbolines (A, B, and M), pyrrolinyl-β-carbolines (G, H, I, P, and Q) and tetrahydro-β-carbolines containing an oxathiazepine ring (C, E, F, K, and L). The latter group displayed the strongest activity against HSV-1 and among



1

		R_1	R_2	R_3
Eudistomin L	**a:**	H	Br	H
K	**b:**	H	H	Br
C	**c:**	H	OH	Br
E	**d:**	Br	OH	H
	e:	H	H	H

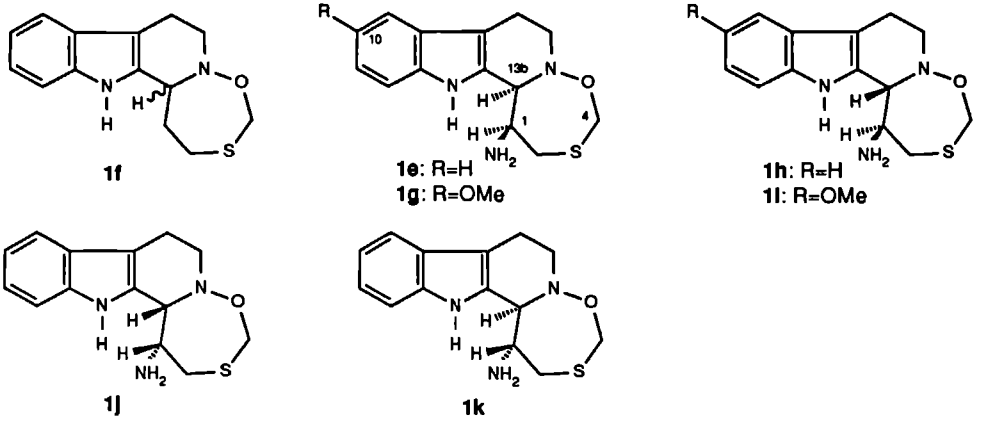
these the order of antiviral activity observed was E (**1d**) (25 ng/disk) > C (**1c**) (50 ng/disk)> L (**1a**) = K (**1b**) (250-400 ng/disk). The potent antiviral eudistomins C and E were active against RNA viruses (Coxsackie A-21 virus and equine rhinovirus) as well as DNA viruses (HSV-1, HSV-2, and *Vaccinia* virus). Acetylation of the amine function of eudistomin C (eudistomin F) abolished the antiviral activity. Antiviral activity has been assayed by using a standard procedure involving monkey kidney cells (CV-1 line).[2] More recently the isolation of eudistomin C, O, K and the new debromoeudistomin K (**1e**) and eudistomin K sulfoxide from the unrelated New Zealand ascodian *Ritterella sigillinoides* has been reported.[3] The activity levels and trends of these compounds were similar to those described above, *i.e.* eudistomin C and K (40-50 ng/disk) > debromoeudistomin K and eudistomin K sulfoxide

(400 ng/disk). These eudistomins exhibited also antiviral activity against *Polio* vaccine Type 1 virus.

These compounds not only display antiviral activity, but as was recently found[3c] for eudistomin K also *in vitro* and *in vivo* antitumour activity against L1210, A549, HCT-8 and P388 cell lines. For P388 the *in vitro* ID_{50} was 0.01 μg/ml whereas an *in vivo* assay gave a T/C value of 137% at 100 mg/kg.

The eudistomins attracted also considerable attention because of their unique chemical structure in combination with their exceptional potent biological activity. The 7-membered oxathiazepine ring -especially the N-O-CH_2-S moiety- is believed to play an important role in this respect. This ring is rarely encountered in natural products and the synthesis of it appeared to be a real challenge. Although the eudistomins were accessible from natural sources in limited amounts, a total synthesis was desirable in order to provide sufficient quantities of the compounds for extensive pharmacological studies and for allowing the preparation of structural analogues. It was not until recently that the total synthesis of the eudistomins was accomplished; two different approaches were reported: the groups of Nakagawa[4] and Still[5] reported an intermolecular Pictet-Spengler reaction as the key-step and the intramolecular approach is described in this thesis (Chapter 7). In addition we were able to synthesize analogues in order to carry out SAR studies. Based on these results attempts will be made to develop a molecule with a higher selectivity for either the antiviral or the antitumour activity. Moreover, we hope to acquire more information about the site of interaction of the title compounds and their working mechanism at a molecular level.

Scheme I



As a first contribution to the realization of these aims, we wish to report here the antitumour activity of **1e-1g** and **1j** (Scheme I) against P388 cells *in vitro* and the antiviral activity of **1e-1k** against a wide variety of viruses including the human immunodeficiency virus (HIV). It should be born in mind that the compounds **1e-1k** tested are not optically pure. Compound **1f** is a racemic mixture and the compounds **1e** and **1g-1k** contain minor amounts (5-7%) of their enantiomers as a result of a low level

of racemization in the reaction sequence (Chapter 7.3).

8.2.2 Antitumour activity.

For a first evaluation of the relation between structure and antitumour activity of the eudistomin derivatives **1e-1g** and **1j**, we used an *in vitro* clonogenic assay of P388 (leukemia) cells.[6] This test is believed to have a high predictive power for *in vivo* activity of the positive compounds. The assyas were performed by P. Lelieveld, TNO-CIVO institutes, Zeist (Netherlands).

Inhibition of P388 colony formation by eudistomins or analogues was determined at several concentrations and the dose causing 50% inhibition of colony formation (ID_{50}) relative to untreated control cells was calculated. The results are collected in Table I.

Table I. Inhibition of P388 Colony Formation.

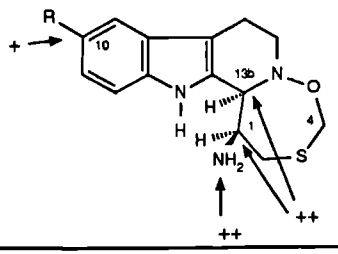
Comp.	ID_{50}	
	(ng/ml)	μM
1e[a]	42	0.15
1f	-[d]	-
1g[b]	3.8	0.012
1j[c]	≈1000[e]	≈3.6

a) Ratio **1e** / **1j** = 94 5 / 5 5 b) Ratio **1g** / enantiomer of **1g** = 93 / 7 b) Ratio **1j** / **1e** = 93 / 7 d) The highest dose tested (729 ng/ml) caused no inhibition e) Predicted value based on the highest tested dose ID_{25} = 527 ng/ml

Discussion

Comparison of the ID_{50} values of the both enantiomers tested (**1e** and **1j**) unambigeously demonstrates that only **1e** -the compound with the "natural" configuration at C(1) and C(13b)- is active. The minor activity observed by **1j** is rather the result of the presence of the enantiomeric impurity (**1e**) which is known to be active. The lower ID_{50} value of the analogue **1g**, which differs from **1e** in having an additional methoxy group at C(10), clearly demonstrates that placing substituents in the indole ring affects the activity of the resulting analogues. This is confirmed by the observed high *in vitro* ID_{50} value (10 ng/ml)[3c] in the P388 assay of eudistomin K (**1b**), which has a bromine

Scheme II. Antitumour activity of eudistomin-analogues.



+ important for activity

atom at the C(10) position of the eudistomin skeleton. Comparison of the ID_{50} value of **1g** (3 8 ng/ml) with that of the well known antitumour compound adriamycine (20 ng/ml)[7] is an indication for the high potency of the eudistomin compounds The lack of activity of **1f** in comparison with **1e**, clearly demonstrates the importance of the amino function at C(1) The results of this preliminary SAR study are summarized in Scheme II.

8.2.3 Antiviral activity

The development of a flexible synthesis for eudistomins allows us to study the structure-activity relationships in some detail The SAR studies on antiviral activity, which have appeared so far[1,3] were hampered by the absence of a total synthesis

This preliminary SAR study focusses on the influence on the activity of the stereochemistry at C(1) and C(13b) and on small alterations of the essential structural moieties

The eudistomins **1e-1k** were evaluated for their inhibitory effects on the replication of a number of viruses, including influenza virus A and influenza virus B in MDCK cells (Table II), respiratory syncytial virus, vesicular stomatitis virus, Coxsackie virus B4 and Polio virus-1 in Hela cell cultures (Table III), parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4 and Semliki forest virus in Vero cell cultures (Table IV), herpes simplex virus type 1 (HSV-1) (strains KOS, F, McIntyre), HSV-2 (strains G, 196, Lyons), the thymidine kinase (TK) deficient (TK) HSV-1 (strain B2006), vaccinia virus and vesicular stomatitis virus in primary rabbit kidney cell cultures (Table V), HIV-1 and HIV-2 in human MT-4 cells (Table VI) The antiviral assays were performed by Prof E. DeClercq, Rega Institute, Leuven (Belgium) Human immunodeficiency virus (HIV) infection was carried out with the HTLV-III_B strain The virus was prepared from the culture supernatant of a persistently HTLV-III_B-infected HUT-78 cell line The antiviral assays were based on inhibition of HIV-induced cytopathogenicity in human MT-4 lymphocytes as described previously [8] The other antiviral assays were based on the inhibition of virus induced cytopathogenicity in either MDCK, HeLa, Vero or primary rabbit kidney cell cultures, following established procedures [9]

Table II Cytotoxic and Antiviral Activity of eudistomin derivatives in MDCK cell cultures

Compound	min cytotoxic concn(MCC)[a], µg/ml	min inhibitory concn[b](MIC), µg/ml	
		influenza virus A (Ishikawa)	influenza virus B (Singapore)
1e	8	0 8	0 8
1f	80	>40	>40
1g	0 8	>0 32	>0 32
1h	15	>8	>8
1i	3	>1 6	>1 6
1j	40	3	3
1k	8	>1 6	>1 6
Ribavirin	>200	15	15

a) Required to cause a microscopically detectable alteration of normal cell morphology b) Required to reduce virus induced cytopathogenicity by 50% Virus induced cytopathogenicity was recorded ar day 5 after infection

As general rule it is accepted that a compound is called antivirally active when the minimal inhibition concentration (MIC) is at least ten-fold lower then the minimal cytotoxic concentration (MCC).

Only, compounds **1e** and **1j** show a ten-fold MIC/MCC ratio and further none of the eudistomin compounds show marked anti-influenza A or influenza B activity at a concentration that is significantly below their toxicity threshold (Table II).

From the eudistomin derivatives, **1g** is effective (MCC/MIC ratios of 13-67) against respiratory syncytial virus, vesicular stomatis virus, Coxsackie virus B4 and polio virus-1 and **1e** against vesicular stomatis virus (MCC/MIC ratio of 20) (Table III).

Table III. Cytotoxic and Antiviral Activity of eudistomin derivatives in Hela cell cultures.

Compound	min. cytotoxic concn(MCC)[a] (μg/ml)	min. inhibitory concn[b](MIC), μg/ml			
		Respiratory syncytial virus (Long)	Vesicular stomatitis virus	Coxsackie virus B4	Polio virus-1
1e	≥4	0.8	0.2	0.55	0.7
1f	≥30	>8	>10	>10	>10
1g	2	0.15	0.03	0.045	0.045
1h	12	>8	>10	>10	>10
1i	4	>1.6	>2.5	>2.5	>1
1j	40	3	4.5	>8.5	>8.5
1k	3	>1.6	>1	>2.5	>2.5
Ribavirin	≥400	3	20	70	110

a) Required to cause a microscopically detectable alteration of normal cell morphology. b) Required to reduce virus-induced cytopathogenicity by 50%. The data represent average values of two separate experiments

Compound **1g** is also specific (MCC/MIC ratios of 10-22) against reovirus type 1, Sindbis virus, Coxsackie virus B4 and Semliki forest virus and **1e** (MCC/MIC ratios of 10-11) against reovirus type 1 and Sindbis virus (Table IV).

Table IV. Cytotoxic and Antiviral Activity of eudistomin derivatives in Vero cell cultures.

Compound	min. cytotoxic concn(MCC)[a,c] (μg/ml)	Minimum inihibitory concn.[b,c] (MIC) (μg/ml)				
		parainfluenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Semliki forest virus
1e	≥4	>0.6	0.4	0.35	0.55	0.45
1f	≥40	>10	>10	>10	>25	>25
1g	≥1	0.55	0.045	0.045	0.07	0.11
1h	25	>7	>10	>10	>10	>10
1i	≥2.5	>1	>0.3	>0.85	0.55	>1
1j	25	7	20	14	7	13
1k	≥10	>4	>4	>4	7	>4
Ribavirin	>400	135	125	>235	>300	>300

a) Required to cause a microscopically detectable alteration of normal cell morphology b) Required to reduce virus-induced cytopathogenicity by 50% c) The data represent average values of two separate experiments

Table V. Cytotoxic and Antiviral Activity of eudistomin derivatives in primary rabbit kidney (PRK) cell cultures.

Compound	min. cytotoxic concn.(MCC)[a,c] (μg/ml)	mininimum inhibitory concn.(MIC)[b,c], μg/ml										
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-1 (F)	Herpes simplex virus-1 (McInyre)	Herpes simplex virus-2 (G)	Herpes simplex virus-2 (196)	Herpes simplex virus-2 (Lyons)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 (B2006) C158/77	Herpes simplex virus-1 (B2006) C137/101	Herpes simplex virus-1 (B2006) ≠3
1e	≥25	1.35	0.25	0.2	0.2	0.2	0.38	0.25	0.2	0.7	0.7	0.85
1f	≥120	>70	>70	>70	>120	>70	>70	>70	>70	>70	>70	>70
1g	≥2.5	0.045	0.039	0.013	0.012	0.014	0.023	0.012	0.015	0.023	0.0085	0.055
1h	≥25		10	>10	>25	>10		>10	>25	>10	>10	>10
1i	≥7	>4	>4	>4	>4	4.5	>2.5	>4	>4	>4	>4	>2.5
1j	≥40	>11	5.4	4.5	4.5	3	4.5	4.5	4.5	>8.5	14	14
1k	≥7	>7	>3	>4	>5.5	2	>2.8	>4	>4	>4	>4	>4
Ribavirin	≥400	>200	>200	>200	>200	>200	>200	30	>400	>200	>200	>200

a) Required to cause a microscopically detectable alteration of normal cell morphology b) Required to reduce virus-induced cytopathogenicity by 50%

c) The data represent average values of two experiments.

The eudistomin derivatives **1e**, **1g** and **1j** are endowed with a marked activity against herpes simplex virus type 1 and 2 (Table V). Especially **1g** shows activies well below the cytoxicity threshold (MCC/MIC ratios of 56-294).

It should be born in mind that the foregoing activities observed for **1j** are rather the result of the presence of the enantiomer impurity (**1e**, 5.5%), which is known to be active.

The anti-HIV-1 and anti-HIV-2 activities and cytotoxicities of the eudistomin analogues are shown in Table VI. Although, in the preceeding tests, **1e** and **1g** proved to be promising antiviral remedies, they failed to give a satisfying therapeutic index against anti-HIV-1 and -2.

Table VI. Anti-HIV-1 and -HIV-2 activity and cytotoxic activity of eudistomin derivatives in human T-lymphocyte (MT-4) cells.

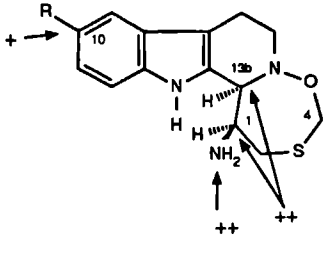
Compound	ED_{50}[a](μM)		CD_{50}[b](μM)	therapeutic index (ratio ID_{50}/ED_{50})	
	HIV-1	HIV-2		HIV-1	HIV-2
1e	>0.116	>0.116	0.27±0.025	<2.3	<2.3
1f	≥154	112±65	370±122	≤2.4	3.33
1g	>0.0066	>0.0066	0.0131	<1.98	<1.98
1h	>5.8	>29	16.4±0.25	<2.8	<0.57
1i	>5.2	>5.2	6.52±3 84	<1.25	<1.25
1j	>5.8	>5.8	13.02±0.04	<2.25	<2.25
1k	>5.8	>5.8	2.79±0.85	<0.48	<0.48
AZT[10]	0.003		4.8	1600	

a) Effective dose of the compound, achieving 50% protection of MT-4 cells against cytopathic effect of HIV
b) Cytotoxic dose of compound, required to reduce the viability of normal uninfected MT-4 cells by 50%

In conclusion, the general outcome of this study is that for the antiviral activities other than the anti-HIV-1 and -2 activity, we observed that: *i)* only the stereoisomers with the same configuration on C(1) and C(13b) as the natural products are active. *ii)* the presence of the amino function at C(1) is of importance. *iii)* substitution in the indole nucleus alters the potency of the compound. For anti-HIV-1 and -2 activity our results indicate that the therapeutic index of the active compounds is unfavourably low.

The results of this preliminary SAR study are summarized in Scheme III.

Scheme III. Antiviral activity of eudistomin-analogues.



\+ important for activity

8.2.4 Epilogue

The strategy for new drug discovery involves two phases: lead identification and structure-activity/toxicity fine tuning. For the eudistomins lead identification is now completed and a start with the second phase has been made. The synthesis developed for the eudistomins enables us to prepare sufficient quantities for further biomedical studies. In addition, more elaborate structure-activity relationship studies will be performed to develop compounds with more selective biochemical and pharmalogical properties.

No data on pharmakinetics or metabolism of eudistomins have been published so far. For further preclinical studies of the eudistomins and analogues evaluation of their pharmacodynamic and biopharmaceutical properties -like metabolism, distribition, excretion, toxicity- in animals is essential.

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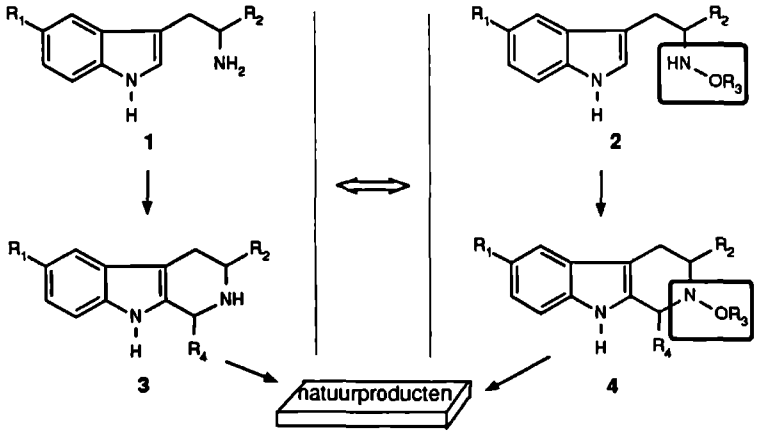
SAMENVATTING

Tryptamines (**1**: R_1=R_2=H) en tryptofaan derivaten (**1**: R_1=H, R_2=COOH) spelen een belangrijke rol in tal van biologische processen, zowel als neurotransmitter in het centraal zenuwstelsel als ook als bouwsteen voor ingewikkelde chemische strukturen, zoals de β-carbolines (**3**)(zie schema I). β-Carbolines staan in de belangstelling vanwege hun breed farmacologisch profiel, zoals blijkt uit de gevonden affiniteit voor tryptamine, serotonine en benzodiazepine receptoren.

Schema I



Dit proefschrift handelt over de synthese van N-hydroxy(alkoxy)-tryptamine (**2**: R_1=R_2=H) en -tryptofaan (R_1=H, R_2=COOEt) derivaten en hun toepassing in de synthese van N-oxy analoga van β-carbolines (**4**) en natuurproducten (zie schema I).

Chemische modificatie van tryptamine- en carboline-structuurfragmenten is onderwerp van intensieve studie. Een snel groeiend aantal derivaten wordt toegepast in de farmacotherapie, met name op het gebied van het centraal zenuwstelsel. De bovengenoemde N-hydroxy- en N-alkoxy-derivaten openen een nieuwe lijn in dit farmacochemisch onderzoek.

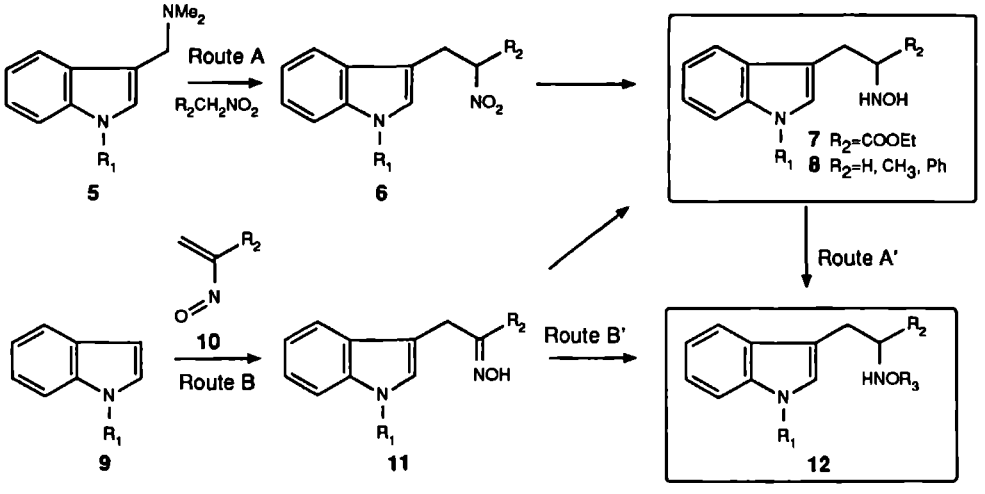
Omdat het zesring N-atoom een sleutelrol vervult bij de farmacodynamische interactie met receptoren, zal het sterk electronegatieve O-atoom de farmacologische eigenschappen van dit type structuren op verrassende wijze kunnen beïnvloeden. Veranderingen in basiciteit, nucleofiliciteit, polarisatie, sterische hindering en extra H-brug vorming moeten in ogenschouw worden genomen. Dit kan enerzijds leiden tot meer informatie op moleculair nivo aangaande de kritische parameters die betrokken zijn bij de receptor affiniteit, en anderzijds tot de ontwikkeling van nieuwe farmacotherapeutica.

In hoofdstuk 2 t/m 7 worden de syntheses beschreven van de N-oxy-tryptamine, -tryptofaan en -β-carboline analoga en in hoofdstuk 8 wordt de biologische activiteit van deze verbindingen in tal van *in vitro* assays vermeld.

In hoofdstuk 2 wordt een efficiënte route naar derivaten van N-hydroxytryptofaan (**7**: R_1=H, R_2=COOEt) en -tryptamine (**8**: R_1=H, R_2=H, Me, Ph) beschreven. Sleutelstap is de reactie van

gramine (**5**) met nitromethaan derivaten, welke resulteert in de corresponderende nitroverbindingen **6**. Reductie van de nitrogroep met Al-amalgam geeft de N-hydroxy verbindingen in hoge opbrengsten (Schema II, Route A).

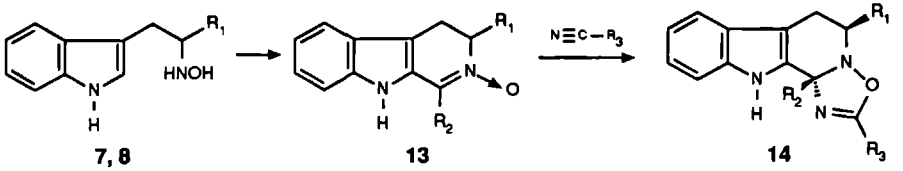
Schema II



Een tweede benadering *via* de oxim verbindingen **11**, welke verkregen werden uit een cycloadditie reactie van indool-derivaten **9** en nitroso-alkenen **10** (Schema II, Route B), was reeds eerder beschreven. Echter, deze route bleek alleen efficiënt te zijn wanneer sterk electron-zuigende groepen (R_2) in **10** aanwezig zijn. Deze route werd gebruikt in een poging om optisch actief N-hydroxytryptofaan te maken. Een diastereoselectieve reductie van de oxim functie van chiraal gesubstitueerde menthol ester derivaten (R_2=COO(-)-8-fenylmenthyl en COO(-)-8-naftylmenthyl) werd gerealiseerd. Helaas was het tot nu toe niet mogelijk om de chirale hulpstof te verwijderen.

N-alkoxy-tryptofaan en -tryptamine derivatives (**12**) zijn gesynthetiseerd door middel van selectieve alkylering van de N-hydroxy verbindingen **7** en **8** (Route A') of door middel van selectieve alkylering van de oxim functie van **11** (Route B').

Schema III

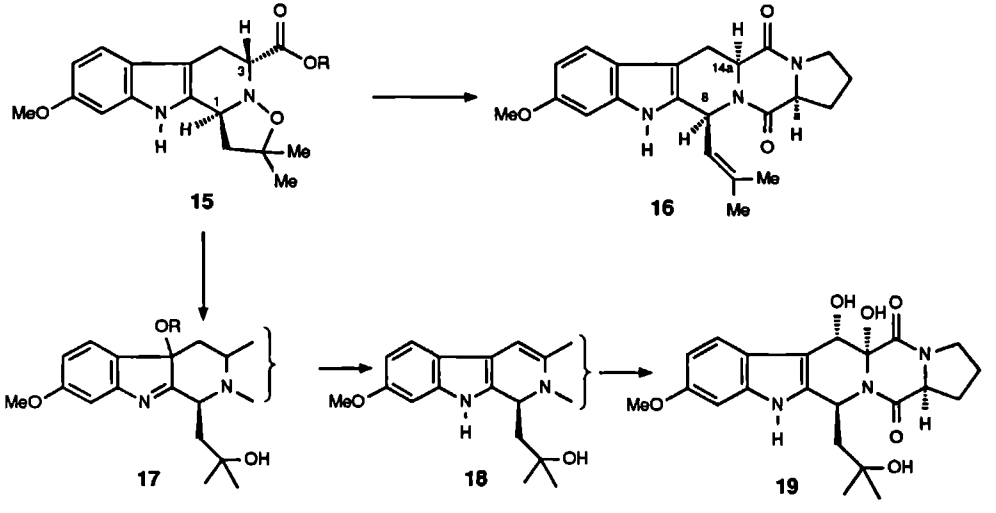


Hoofdstuk 3 gaat over de synthese van 3,4-dihydro-β-carboline nitronen **13**, waarbij wordt

uitgegaan van de corresponderende N-hydroxy-tryptofaan of -tryptamine derivaten (Schema III). Vervolgens wordt de 1,3-dipolaire cycloadditie besproken van deze nitronen met nitrillen, een reactie die resulteert in de Δ^4-1,2,4-oxadiazolines (**14**).

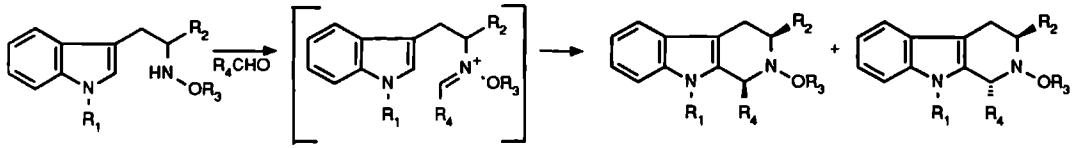
In hoofdstuk 4 wordt de toepassing van isoxazolidine **15** besproken in de totaal-synthese van fumitremorgine C (**16**). In het tweede gedeelte van dit hoofdstuk wordt de totaal-synthese van een tweede lid van deze familie van mycotoxines besproken d.w.z. verruculogen TR-2 (**19**). In deze synthese werd de formele dehydrogenering van tetrahydro-β-carbolines **15** tot 3,4-dehydro-β-carbolines (**18**) bewerkstelligd waarbij 3-alkoxyindolenine derivaten (**17**) als tussenproduct optreden. De *cis*-diol functie in **19** werd verkregen door hydroxylering van de dubbele band met osmium tetroxide (Schema IV).

Schema IV



In hoofdstuk 5 wordt de invloed besproken van de substituenten R_1-R_3 in N-oxy-tryptofaan en -tryptamine derivaten op hun reactiviteit in de intermoleculaire Pictet-Spengler reactie met aldehydes (R_4CHO) resulterend in *cis*- (**21**) en *trans*-β-carbolines (**22**) (Schema V). Aandacht werd

Scheme IX

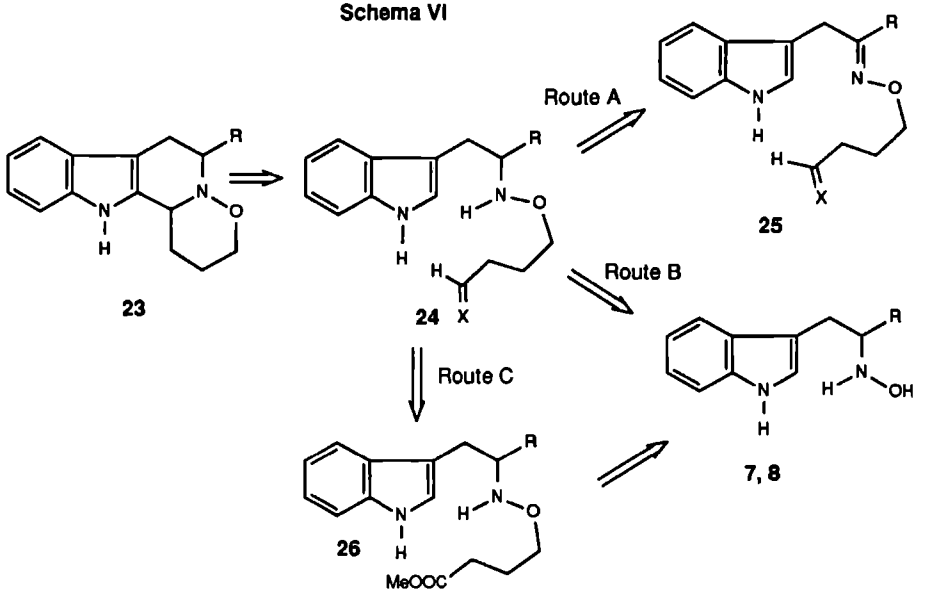


besteed aan factoren die de *cis/trans* verhouding beïnvloeden. Tengevolge van het toegenomen electrofiele karakter van de C=N dubbele band in intermediair **20**, verloopt de reactie sneller en

minder specifiek dan de reactie met de deoxy analoga.

Hoofdstuk 6 behandelt de synthese van tetracyclische N-oxo-β-carbolines **23** (R=H, CH_3, COOEt), analoga van indoolalkaloiden van het corynantheine type. Deze doelmoleculen werden verkregen door middel van een nieuwe intramoleculaire Pictet-Spengler reactie van tussenproduct **24** (Schema VI). Dit belangrijke tussenproduct is een N-alkoxytryptamine met een aldehyde functie of een

Schema VI



gemaskeerd aldehyde functie op de δ-positie van de alkoxy zijketen. De toegankelijkheid van dit intermediair werd langs drie wegen bestudeerd:

i) Reductie van een O-gealkykeerde oxim functie aanwezig in **25** (Route A).

ii) Selectieve O-alkylering van de N-hydroxy-functie van verbindingen **7** of **8** met een gefunctionaliseerd vier-koolstof substraat (Route B).

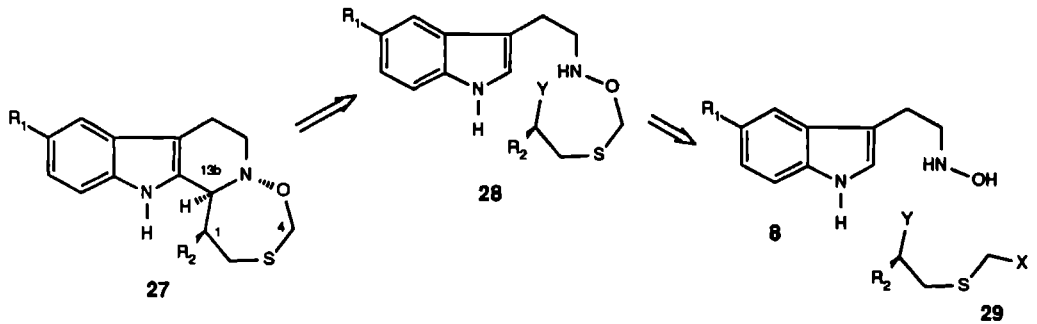
iii) Selectieve reductie van de ester functie aanwezig in **26**, verkregen uit **8**.

Het meest efectief waren route-B en -C, terwijl route-A slecht verliep.

In hoofdstuk 7 worden resultaten beschreven verkregen door toepassing van de intramoleculaire Pictet-Spengler reactie in de synthese van een eudistomine derivaat en enkele analoga daarvan. Intramoleculaire reactie van **28** (R_1=H of OMe, R_2=H of NH_2, Y=$CH(OMe)_2$ of COOMe) -verkregen uit **8** en het chloormethylsulfide derivaat **29**- gaf toegang tot de eudistomines **27** (Schema VII).

Hoofdstuk 8 gaat over structuur-activiteit relatie studies. Het eerste gedeelte van dit hoofdstuk beschrijft de affiniteiten van de N-hydroxy(alkoxy)-tryptofaan en -tryptamine derivaten, de 3,4-dihydro-N-oxo-β-carbolines, de N-oxo-tetrahydro-β-carbolines en de tetracyclische N-oxo-tetrahydro-β-carbolines voor de tryptamine-, serotonine - en benzodiazepine-receptoren. In

Schema VII



het algemeen is de affiniteit van de N-oxo-verbindingen minder dan die van de overeenkomstige desoxo verbindimg, maar daar tegenover staat dat sommige van de eerstgenoemde verbindingen een verhoogde selectiviteit bezitten.

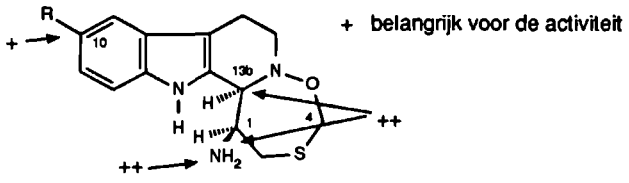
Het tweede gedeelte van dit hoofdstuk beschrijft de structuur-werkingsrelatie van de gesynthetiseerde eudistomines met betrekking tot antivirale en antitumor activiteit.

Deze eerste SAR-studie beperkt zich tot het belang van de stereochemie der chirale centra C(1) en C(13b) voor de antivirale activiteit. Bovendien wordt de invloed van twee andere structurele parameters bestudeerd. Zeven gesynthetiseerde eudistomine-analoga (**27**) werden getest op hun vermogen de replicatie van tal van virussen te remmen, waaronder de "human immunodefiency virus" HIV-1 en HIV-2.

Voor alle virussen, met uitzondering van HIV-1 en HIV-2 werd gevonden dat *i)* alleen stereoisomeren, die dezelfde configuratie op C(1) en C(13b) bezitten als het natuurproduct zelf actief zijn *ii)* de aanwezigheid van de aminofunctie op C(1) van belang is *iii)* door het aanbrengen van substituenten in de indoolkern de activiteit van de resulterende verbinding verandert.

Vier van de gesynthetiseerde eudistomine-analoga werden onderzocht op hun activiteit tegen tumor cellen (P388 leukemie) in een *in vitro* assay. Onze voorlopige gegevens lieten een grote overeenkomst zien met de gegevens verkregen in de bepaling van de antivirale activiteit der stoffen, inzoverre het gelijke structuurparameters betreft. De resultaten van beide SAR-studies zijn schematisch weergegeven in schema VIII.

Schema VIII. Antivirale en antitumor activiteit van eudistomine analoga



De actiefste stof is verbinding **27** waarin R_1=OMe en R_2=NH_2. Dit analogon is vijf maal actiever, in de gebruikte assay, dan de bekende antitumorverbinding adriamycine.

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2. *"Nitrone Cycloaddition in the Stereoselective Synthesis of β-Carbolines from N-hydroxy tryptophan."*
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7. *"1,3-Dipolar Cycloaddition of Nitrones with Nitriles. Scope and Mechanistic Study."*
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11. *"Crystal structure determination of rel-(6R, 12bR)-6-ethoxycarbonyl-1,2,3,6,7,12,12b-heptahydro-[1,2]oxazino [2',3':1,2]pyrido[3,4-a]indole, $C_{17}H_{20}N_2O_3$."*
    Pedro H.H. Hermkens, Jan H. v. Maarseveen, J.M.M. Smits, Paul T. Beurskens, *J.Crystallogr.Spectrosc.Res.* submitted.
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stereoisomers."
Pedro H.H. Hermkens, Jan H. v. Maarseveen, Harry C.J. Ottenheijm, Chris G. Kruse, Hans W. Scheeren, *J.Org.Chem.* in press.

13. *"Crystal structure determination of (1S, 13bR)-1-amino-10-methoxy-1,2,7,8,13,13b-hexahydro-[1,6,2]oxathi azepino[2' 3':1,2]pyrido[3,4-a]indole, $C_{15}H_{19}N_3O_2S.H_2O$."*
Pedro H.H. Hermkens, Jan H. v. Maarseveen, W.P. Bosman, J.M.M. Smits, Paul T. Beurskens, *J.Crystallogr.Spectrosc.Res.* submitted.
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Pedro H. Hermkens, Chris, G. Kruse, Hans W. Scheeren, Harry C.J. Ottenheijm, *J.Org.Chem.* submitted.
15. *"Structure-activity relationship studies of N-hydroxy(alkoxy)tryptamine derivatives on the serotonin- and tryptamine receptors."*
Pedro H.H. Hermkens, Jan H. v. Maarseveen, Martin Th. M. Tulp, Hans W. Scheeren, Chris G. Kruse, in preparation.
16. *"Structure-activity relationship studies of eudistomin derivatives on antitumour and antiviral activities.:*
Pedro H.H. Hermkens, Jan H. v. Maarseveen, Peter Lelieveld, Eric De Clercq, Jan Balzarini, Chris G. Kruse, Hans W. Scheeren, in preparation.

Abstracts of papers presented at international meetings:

1. The first Belgian Organic Synthesis Symposium, Namur, Belgium, 1986, *"A nitrone derived from N-hydroxytryptophan in the strategy of natural product synthesis"*. Pedro H. H. Hermkens, Ralf Plate, Harry C.J. Ottenheijm. *(Poster)*
2. 15th IUPAC International Symposium on the Chemistry of Natural Products Den Haag, The Netherlands, 1986, *"A nitrone derived from N-hydroxytryptophan in the strategy of natural product synthesis"*. Pedro H. H. Hermkens, Ralf Plate, Harry C.J. Ottenheijm. *(Poster)*
3. 11th International congress of Heterocyclic Chemistry (ICHC), Heidelberg, Federal Republic of Germany, 1987, *"First total Syntheis of Fumitremorgin C and an approach to verruculogen TR-2."* Pedro H.H. Hermkens, Ralf Plate, Harrie C.J. Ottenheijm. *(Poster)*
4. 31st National Organic Chemistry Symposium (NOS) of the American Chemical Society, Cornell University, Ithaca, USA, 1989, *"Intramolecular Pictet-Spengler reaction. An approach to N-alkoxy-tetrahydro-β-carbolines."* Pedro H.Hermkens, Jan H. v, Maarseveen, Chris G. Kruse, Hans W. Scheeren. *(Poster)*
5. 12th International congress of Heterocyclic Chemistry (ICHC), Jerusalem, Israel, 1989. *"Intramolecular Pictet-Spengler reaction. An approach to N-alkoxy-tetrahydro-β-carbolines."* Pedro H.Hermkens, Jan H. v, Maarseveen, Chris G. Kruse, Hans W. Scheeren. *(Lecture)*

CURRICULUM VITAE

Pedro H.H. Hermkens werd geboren op 1 februari 1958 te Asten. In 1975 behaalde hij het HAVO-diploma aan het Titus Brandsma Lyceum te Oss. In hetzelfde jaar startte hij met de studie H.T.S.-chemie aan de I.H.B.O. te Eindhoven. In juni 1979 slaagde hij voor deze H.T.S.-opleiding.

Vanaf september 1979 tot januari 1981 was P.H. werkzaam als analist op de Technische Hogeschool te Eindhoven bij de vakgroep Organische Chemie onder leiding van Prof. Dr. H.M. Buck. In deze periode volgde hij tevens de part-time MO-A opleiding Scheikunde-Natuurkunde aan de Katholieke Leergangen te Tilburg. In juni 1981 sloot P.H. deze opleiding met succes af.

Vanaf januari 1981 tot en met juni 1981 heeft hij als leerkracht aan de Scholengemeenschap 'De Ruivert' te Oss gewerkt.

Vanaf september 1981 studeerde hij scheikunde aan de Katholieke Universiteit te Nijmegen. Het kandidaatsexamen (S1) behaalde hij op 27 juni 1983. De doctoraal studie omvatte als hoofdrichting Organische Chemie (Dr. H.C.J. Ottenheijm, onderwerp: Nitroncycloaddities in de synthese van indoolalkaloiden.), een bijvak Farmacochemie (Prof. Dr. J.M. v. Rossum, onderwerp: De farmacokinetiek van nicotine en cotinine en de invloed van orale contraceptieve steroiden) en een bijvak Biochemie (Prof.Dr. H.P.J. Bloemers, onderwerp: Het bereiden van een rattebank m.b.v. cosmide vectoren met als doel een variant van het kattesarcoma virus te gaan cloneren). Het behalen van het doctoraalexamen op 25 november 1985 vormde de afsluiting van deze fase.

Vanaf 16 januari 1986 tot 31 december 1989 was P.H. werkzaam als wetenschappelijk medewerker binnen de vakgroep Organische Chemie (Prof. Dr. B. Zwanenburg; Dr H.C.J. Ottenheijm) van de Katholieke Universiteit te Nijmegen.

In deze periode werd aanvankelijk onder leiding van Dr. H.C.J. Ottenheijm en na diens overgang naar Organon onder leiding van Dr J.W. Scheeren, op een project van de Stichting voor de Technische Wetenschappen (STW), het in dit proefschrift beschreven onderzoek uitgevoerd. Vanuit Duphar B.V., de utilisatiepartner in dit project, was Dr. C.G. Kruse zijn begeleider.

Onderzoeksresultaten werden gepresenteerd op diverse nationale en internationale bijeenkomsten en congressen.

Sinds 31 december 1989 is hij werkzaam bij Organon International B.V. te Oss.